Conformational States of *myo*-Inositol Hexakis(phosphate) in Aqueous Solution. A ^{13}C NMR, ^{31}P NMR, and Raman Spectroscopic Investigation

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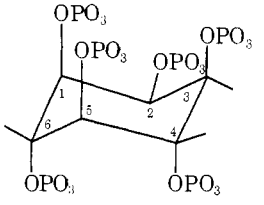
Abstract: The longstanding question of the solution conformation of *myo*-inositol hexakis(phosphate) (IHP) has been resolved through combined use of NMR and Raman spectroscopic methods. IHP exists in aqueous solution in either of two conformations, depending on pH: the low-pH form possesses one axial and five equatorial phosphates (1-ax/5-eq), whereas the high-pH form has the inverted 5-ax/1-eq structure. Equal amounts of the two conformers coexist at pH 9.4 ± 0.1 for 0.10 M Na_{12}IHP at 27 °C. The ^{13}C NMR spectra of IHP (recorded over the pH range 0.5–12.5) are relatively insensitive to phosphate deprotonation, but do exhibit a single, major change in appearance centered around pH 9.4; this provides strong support for a conformational change. The ^{31}P NMR spectra as a function of pH reveal, among other things, that the three least acidic phosphate protons have apparent pK_a values of 9.2–9.6. The IHP ring inversion is most probably triggered by one or more of these acid-dissociation steps. The Raman spectra are sensitive both to acid dissociation and conformation, and also allow key spectral comparisons between aqueous solutions of IHP and solids of known structure (viz., solid Na_{12}IHP having 5-ax/1-eq phosphates and solid *myo*-inositol having 1-ax/5-eq hydroxyls). Raman data indicate that alkali metal ions preferentially bind to, and thus stabilize, the aqueous high-pH IHP conformer in the order $Li^+ \sim Na^+ > Cs^+$. The Raman spectrum of solid Ca_6IHP is characteristic of the 1-ax/5-eq conformer, in contrast to the 5-ax/1-eq structure of the dodecasodium salt.

myo-Inositol hexakis(phosphate) (designated IHP or phytate) is, for two principal reasons, a frequent subject of biochemical investigation. First, there has long been interest in determining the extent to which phytate, the major phosphorus-containing constituent of cereal grains, decreases the nutritional availability of dietary metal ions (e.g., calcium, iron, and zinc) through complexation.[1] Second, researchers have found that, owing to its very tight binding to deoxyhemoglobin, phytate is one of the most effective chemical agents for decreasing the affinity of hemoglobin for oxygen.[2]

In spite of this research interest, a clear picture of phytate's molecular structure has been slow to develop. Early controversy centered around the conflicting structures proposed by Neuberg[3] and Anderson,[4] Neuberg's being a hydrated tris(pyrophosphate) derivative of *myo*-inositol and Anderson's the hexakis(phosphate). Only within the last decade was Anderson's IHP structure shown through a ^{31}P NMR analysis to be correct;[5] the exact solution *conformation,* however, was left unproven. These and subsequent NMR workers[6] appear to have assumed that fully deprotonated (sodium) IHP in aqueous solution possesses the conformation in which one phosphate is axial and five are equatorial (1-ax/5-eq). Other workers,[7] however, thought it likely that the solution contains the inverted 5-ax/1-eq conformer shown below (in both IHP conformations, the unique substitution is on carbon atom numbered 2, C-2). This 5-ax/1-eq form had been found using X-ray diffraction for IHP in its hydrated dodecasodium salt.[7] The 5-ax/1-eq conformation was also postulated for totally protonated aqueous IHP on the basis of potentiometric measurements.[8] Overall, the work to date on IHP's solution conformation has been far from conclusive.



We have now resolved this longstanding structural problem through combined use of ^{13}C NMR, ^{31}P NMR, and Raman spectroscopy: IHP exists in aqueous solution as the 1-ax/5-eq conformer at low pH and the 5-ax/1-eq conformer at high pH. ^{13}C NMR gave information about the ring conformation, while ^{31}P NMR provided details of IHP's acid-dissociation processes. Raman spectroscopy was responsive to both acid dissociation and ring conformation and, in addition, allowed key spectral comparisons between aqueous solutions and corresponding solids of known structure.

Experimental Section

Materials. *myo*-Inositol (Aldrich Chemical Co.)[9] and hydrated Na_{12}IHP (Sigma Chemical Co., Lot 72C-1180) were used as received. The previously published crystal structure of Na_{12}IHP had been determined using Sigma Chemical Co. material, purified by recrystallization from water.[7] We found the Raman spectrum of freshly recrystallized Sigma Na_{12}IHP to be basically the same as that of the unrecrystallized Sigma solid used in our spectroscopic investigation. The small spectral variations that we did observe probably arose only from a difference in crystalline hydration level, since both solids, when dissolved in water, produced exactly the same Raman spectrum. The (hydrated) hexacalcium salt of IHP was allowed to precipitate from a pH 8 solution of $CaCl_2$ and Na_{12}IHP as described in the literature;[10] the Ca:P atom ratio of this material was analyzed as 0.98 (calcd, 1.00). All other chemicals were reagent grade; alkali-metal chlorides were dried at 105 °C before use.

Solutions were prepared using standard gravimetric and volumetric techniques and, for Raman analysis, passed through a Millipore filter prior to spectral recording. Aliquots of stock Na_{12}IHP solutions were brought to the desired pH through addition of small amounts of HCl or NaOH solutions. *Hereafter in this paper, Na_{12}IHP solutions will simply be called sodium IHP solutions.*

NMR Measurements. Proton-decoupled ^{13}C NMR spectra were recorded on a Varian CFT-20 spectrometer operating at 20 MHz with deuterium stabilization. The free-induction decays were digitized to 8K data points with a cycle time of 1 s. Ca. 30 000 scans were averaged for each spectrum. A pulse width corresponding to a 40° flip angle was used. Field-frequency stabilization was maintained through use of a D_2O-containing capillary centered with a Teflon plug in each 10-mm sample tube. The ^{13}C resonance positions were referenced to the resonance of a secondary standard, 1,4-dioxane, which was dissolved in the D_2O. All chemical shifts are reported relative to tetramethylsilane ($\delta_{Me_4Si} = \delta_{dioxane} + 67.4$ ppm); owing to the breadth of the ^{13}C resonances, chemical shifts are considered accurate to only ±4 Hz. In those cases where the dioxane peak overlapped the IHP signals, two spectra were recorded, the first with a D_2O/dioxane capillary and the second with a capillary containing only D_2O. ^{13}C spectra were recorded at 37 °C.

Proton-decoupled ^{31}P NMR spectra were recorded on a modified Bruker HX-90 spectrometer[11] operating at 36.4 MHz with fluorine

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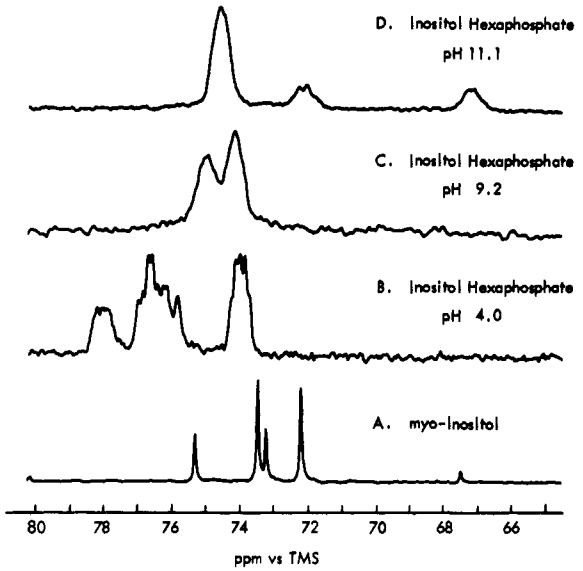

Figure 1. ^{13}C NMR spectra of aqueous solutions containing (A) saturated *myo*-inositol in D_2O; (B) sodium IHP at pH 4.0; (C) sodium IHP at pH 9.2; (D) sodium IHP at pH 11.1. The peak at 67.4 ppm in the spectrum of *myo*-inositol is due to the 1,4-dioxane standard. For presentation here, IHP spectra were recorded without any standard. All IHP solutions were 0.10 M.

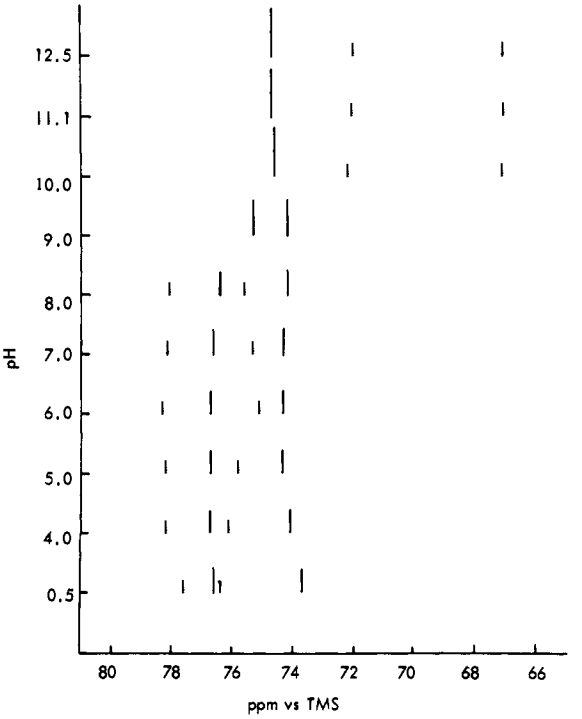

Figure 2. Spectral line positions for the ^{13}C NMR spectra of 0.10 M aqueous sodium IHP solutions as a function of pH. Relative peak areas are represented by the height of the bars.

stabilization. A Nicolet 1080 computer was used for signal averaging and Fourier transformation. The free-induction decays were digitized to 4K or 8K data points with a cycle time of 2 or 4 s. Ca. 2000–4000 scans were averaged for each spectrum. A pulse width corresponding to a ~30° flip angle was used. Field-frequency stabilization was maintained through use of a C_6F_6-containing capillary centered with a Teflon plug in each 10-mm sample tube. The ^{31}P resonance positions were referenced externally to the ^{31}P resonance of 85% H_3PO_4. Positive ^{31}P chemical shifts correspond to resonances downfield from the H_3PO_4 reference signal. For well-resolved resonances, the chemical-shift accuracy is considered to be ±0.5 Hz; for overlapping resonances in the pH 5–10 range, the accuracy is worse. ^{31}P spectra were recorded at 32 °C.

Raman Measurements. Raman spectra were recorded on a Spex Ramalog 5 spectrometer using the 514.5-nm line from a Coherent Model CR-3 Ar^+ laser as excitation source and a cooled C31034 photomultiplier tube for photon-counting detection. The laser power at the sample typically was ca. 350 mW, and the spectral slit widths at 514.5 nm were ca. 8 cm^{-1} for solutions and ca. 4 cm^{-1} for solid samples. The frequency scale was calibrated using CCl_4 and C_6H_6; except for very broad bands and shoulders, positions of band maxima are considered accurate to ±2 cm^{-1}. The 90° excitation-viewing mode was used to obtain spectra of solutions contained in sealed 1-mm i.d. glass capillaries and of solids that either covered the inside bottom surface of a vial or were packed into a conical depression at the end of a metal rod. The temperature of aqueous solutions in the laser beam was 27 °C. Depolarization ratios were measured by conventional methods; for this purpose a liquid cell with flat windows for laser beam entrance and exit was used.

Results and Discussion

^{13}C NMR Spectra. Figure 1 displays the ^{13}C NMR spectra of 0.10 M solutions of sodium IHP at representative pH values of 4.0, 9.2, and 11.1, and, for comparison, the spectrum of the parent hydroxylated compound, *myo*-inositol. The breadth and, in some cases, the complex splitting of the IHP signals arise from C–P spin–spin coupling interactions. A crystal-structure analysis of solid *myo*-inositol showed that the hydroxyl groups adopt the 1-ax/5-eq arrangement.[12] In the absence of evidence to the contrary, this structure has traditionally been assumed for the *myo*-inositol aqueous solution species as well. During the course of our investigation, we made a direct Raman comparison of the solid and solution *myo*-inositol structures, a comparison that showed without question that, indeed, it is the 1-ax/5-eq form that exists in solution (vide infra). We therefore consider the spectrum in Figure 1A, with a 1:2:1:2 pattern of relative integrated intensities, to be characteristic of the 1-ax/5-eq conformation. The ^{13}C NMR spectrum of *myo*-inositol has been previously reported and assignments have been made based upon comparison with spectra of isomeric and methyl-substituted inositols.[13] The four resonances in Figure 1A are assigned accordingly: from low to high field, C-5; C-1,3; C-2; and C-4,6.

The spectrum of *myo*-inositol can be compared with the spectrum of IHP at low pH (e.g., pH 4.0, Figure 1B). Replacement of each hydroxyl group in *myo*-inositol with a phosphate group is expected to have a deshielding shift effect of nearly equal magnitude for each carbon atom, provided that the conformational state is preserved. The spectra of IHP at low pH show four signals with relative integrated intensities of 1:2:1:2, shifted downfield relative to the *myo*-inositol lines. The similarity between Figures 1A and 1B leads us to conclude that IHP at low pH has the 1-ax/5-eq arrangement of phosphate groups. Our Raman data support this conclusion.

The ^{13}C NMR spectra of IHP at pH 9.2 (Figure 1C) and 11.1 (Figure 1D) are different from each other and also quite different from that at pH 4.0 (Figure 1B). We get a better overall picture of this spectral transition in Figure 2; here we use a stick format for simplicity, with the stick heights representing relative integrated intensities. The 1:2:1:2 intensity pattern that we have associated with the 1-ax/5-eq conformation is found over a wide pH range (0.5–8), a range in which one or two protons dissociate(s) from each phosphate group (see later discussion on pK_as). The relative insensitivity of these ^{13}C NMR spectra toward deprotonation equilibria[14] indicates that the *major* observed spectral change, from a 1:2:1:2 pattern for the 1-ax/5-eq conformer at low pH eventually to a 4:1:1 pattern at high pH, must arise from a ring conformational change. As one might expect, the high-pH conformer is the 5-ax/1-eq form, as will be proven by our Raman data. The ^{13}C spectra at intermediate pH (e.g., pH 9 in Figure 2) are interpreted as the average of the spectra of the two rapidly interconverting chair conformations, weighted according to relative populations.

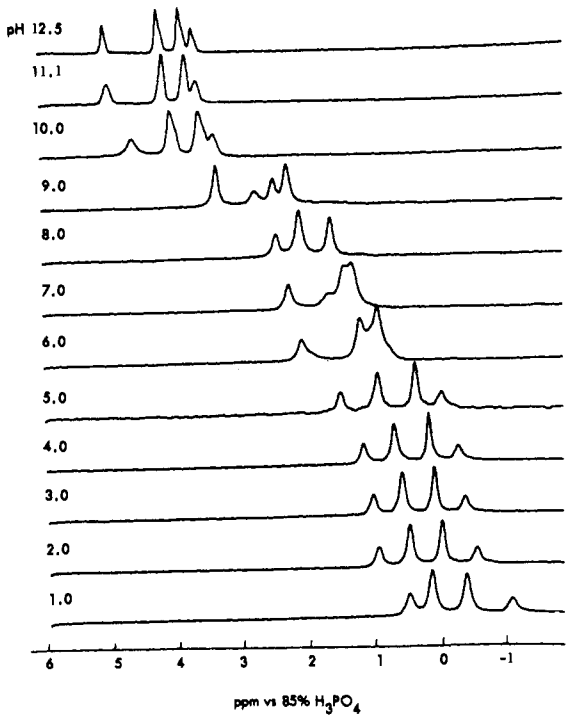

Figure 3. ^{31}P NMR spectra of 0.10 M aqueous sodium IHP solutions at representative pH values.

Tentative assignments for the 4:1:1 IHP spectra at pH 10.0 (and above) can be made on the basis of relative intensities by invoking accidental chemical-shift equivalence for C-1,3 and C-4,6. Of the two higher field resonances, the higher is assigned to C-5 and the lower to C-2; these assignments best account for the chemical shifts in the exchange-averaged intermediate-pH spectra.

^{31}P NMR Spectra. We recorded ^{31}P NMR spectra of IHP as a function of pH (Figure 3) in order to determine apparent acid-dissociation pK_as under the specific conditions of our ^{13}C NMR and Raman experiments (i.e., ca. 0.10 M IHP, Na^+ counterions). (By *apparent* pK_as, we mean mixed activity-concentration quotients.) These pK_a values helped us to better understand the acid-dissociation processes accompanying the ring conformational interconversion. A previous potentiometric study had shown that IHP (ca. 0.01 M, Na^+ counterions) possesses six strong acid sites with $pK_a \sim 1.8$, two moderately weak acid sites with $pK_a \sim 6.3$, and four very weak acid sites with $pK_a \sim 9.7$.[8] The pK_a values 1.8 and 6.3 are typical of simple phosphoric acid monoesters,[15] but the pK_a value 9.7 is unusually high, no doubt reflecting the hydrogen-bonding interactions that surely exist among the phosphate groups in IHP. A recent ^{31}P NMR investigation of IHP (0.010 M, *tetrabutylammonium* counterions) led to pK_a values of 1.1–2.1 (six protons), 5.7 (one proton), 6.8–7.6 (two protons), and 10–12 (three protons).[6] The effect on the pK_as of varying the countercation will be discussed more fully later.

The downfield displacement of the ^{31}P resonances of IHP with increasing pH is typical of phosphates and results largely from electronic and phosphate-geometry effects accompanying deprotonation.[16] (There is probably a lesser ^{31}P chemical-shift dependence on ring conformation.)[16] For any resonance at a particular pH, the chemical shift is the weighted average of shifts for the appropriate protonated and deprotonated forms. In order to determine pK_as, we used the well-established procedure[16] of tracking the chemical shift of each resonance over a wide pH range (viz., 0.5–12.5), a procedure made difficult with IHP by extensive signal overlap between pH 6 and 9. To assist in following the bands through this region of overlap, we will briefly consider spectral assignments. Adopting the assignments of earlier workers,[5] we attribute the four resonances of the high-pH 1:2:2:1 spectrum (from low to high field) to phosphates on C-2, C-4 and 6, C-1 and 3, and C-5. It appeared to us that, as the pH is lowered from 12.5 to 0.5, the chemical-shift degeneracy of the C-1,3 and C-4,6 phosphorus signals is preserved, as is the chemical-shift uniqueness of the C-2 and C-5 phosphorus resonances. Our best attempt at tracking the resonances led to assignments for the low-pH 1:2:2:1 spectrum that differ from the high-pH assignments only in that positions of resonances for C-1,3 and C-4,6 phosphates are reversed.

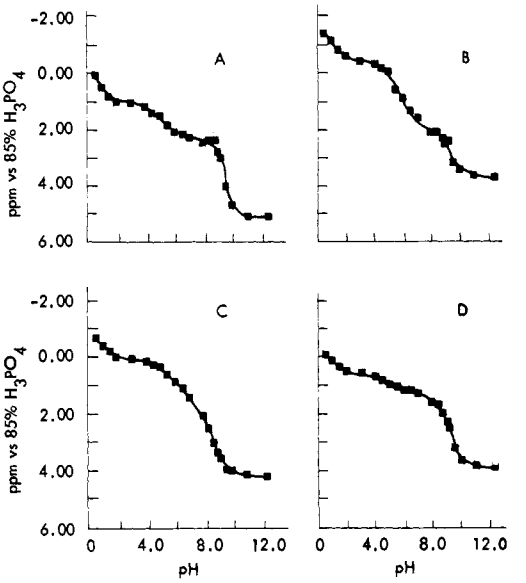

Figure 4. ^{31}P NMR chemical-shift titration plots for IHP phosphate groups: (A) on C-2; (B) on C-5; (C) on C-4 and 6; (D) on C-1 and 3.

This tracking procedure led to the plots shown in Figure 4; the apparent pK_as are the pH values at the points of inflection. Under the conditions of our experiments, the six most acidic protons, one on each phosphate group, dissociate with pK_as that appear to fall in the normal range of 1–2. As for loss of the remaining six protons, the number of inflections suggests that extensive proton sharing exists in the various IHP anions having the 1-ax/5-eq structure. Thus, the chemical shift for the phosphorus atom on C-2 (Figure 4A) and on C-5 (Figure 4B) *each* is affected by the loss of *two* additional protons: for C-2, the pK_as are 5.2 and 9.6; for C-5, they are 6.0 and 9.4. The chemical shift for the two phosphorus atoms on C-4 and C-6 (Figure 4C) also appears to be affected by the loss of two additional protons ($pK_a \sim 6.0$ and 8.6), as is the chemical shift for the phosphorus atoms on C-1 and C-3 (Figure 4D, $pK_a \sim$ 5.0 and 9.2). We believe, however, that, in the C-4,6 and C-1,3 titration plots, the poorly defined inflection at the lower pH results from the influence of deprotonation of the C-5 and C-2 phosphate groups, respectively.[17] The entire set of pK_a values determined here agrees fairly well with those found by other workers potentiometrically.[8] The important point to note is that dissociation of the (three) least acidic protons (pK_a = 9.2–9.6) accompanies the crossover in appearance of the ^{13}C NMR spectra, the crossover indicative of a conformational change.

Raman Spectra. Our Raman data corroborate the existence of a pH-dependent conformational change in aqueous IHP solution and aid in assigning molecular structures to the species involved. Of the many Raman spectra recorded of aqueous sodium IHP over the pH range 0.8–12.5, three that clearly illustrate the conformational transition (viz., those of 0.10 M solutions at pH 8.5, 9.4, and 10.0) are presented here (Figures 5B–D). Comparison among these spectra and with those of aqueous *myo*-inositol (Figure 5A) and the solid dodecasodium salt of IHP (Figure 5E) will serve to support our major con-

clusions. Although the most useful spectral region for this analysis is that displayed in Figure 5 (650–1450 cm^{-1}), the frequencies of C–H stretching bands near 2950 cm^{-1} appear to correlate with IHP conformation and are also discussed below. At frequencies lower than those shown in Figure 5, the deformation bands of aqueous IHP are badly overlapped by the broad water libration band near 450 cm^{-1} and do not relate in any obvious fashion to IHP conformation.

Since Raman spectra of IHP have not, to our knowledge, been previously reported in the literature, we will comment briefly on our assignment of several of the bands in Figure 5. The bands lying between 1250 and 1400 cm^{-1}, all depolarized in the solution spectra, are assigned to C–C–H and O–C–H bending vibrations (we will henceforth refer to these simply as C–H bending vibrations). Similar Raman band assignments have been firmly established for several carbohydrate molecules.[18] This region of the spectrum contains negligible contribution from stretching vibrations localized within IHP's six phosphate groups; even the highest frequency P–O band observed, that at ca. 1200 cm^{-1} arising from phosphoryl stretching of the fully protonated PO_4H_2 groups (at pH ~0.8), falls outside this range. In the spectrum of *myo*-inositol in D_2O (Figure 5A), moreover, several C–O–H deformation bands that normally also lie in or near the 1250–1400-cm^{-1} range have been shifted to lower frequency through deuterium exchange with the solvent. Comparison of Figures 5A and 5E points out the dependence of the C–H bending bands on ring conformation: the 1-ax/5-eq arrangement (of hydroxyls) in *myo*-inositol[19] gives rise to maximum intensity near 1380 cm^{-1}, whereas the 5-ax/1-eq arrangement (of phosphates) in the sodium IHP solid produces greatest intensity near 1300 cm^{-1}.

The unequivocal assignment of many of the remaining bands in Figure 5 is hampered by expected coupling among C–C, C–O, and, for the IHP samples, P–O stretching motions. These bands should be sensitive to both proton dissociation and ring conformation. Our assignment of these bands is discussed in the supplementary material of this paper.[20]

It is in the unobscured C–H bending region, between 1250 and 1400 cm^{-1}, where a pH-dependent change in IHP conformation is most clearly indicated: as the pH is raised from 8.5 to 10.0, the pattern of bands at 1267, 1291, 1313, 1352, and 1374 cm^{-1} gradually transforms into a quite different pattern at 1284, 1303, 1326, 1372, and 1384 cm^{-1}. The former pattern of intensities appears in all spectra of IHP solutions more acidic than pH 8.5, although the frequencies of the entire five-band set do shift upward by ca. 10 cm^{-1} as pH 0.8 is approached. This nearly invariant appearance indicates that the C–H bending vibrations are directly affected only little by the phosphate ionization state, since PO_4H_2, PO_4H^-, and PO_4^{2-} groups all exist in the pH 0.8–8.5 range. The pH 10 band pattern, moreover, is exactly reproduced at pH values as high as 12.5. The interconversion of these two distinct sets of bands must reflect a transition between two IHP conformations having quite different dispositions of C–H bonds.

The close overall similarity of the spectra in Figures 5D and 5E establishes the identity of the high-pH solution conformation as that possessing the 5-ax/1-eq arrangement of phosphates. Likewise, the similarity of C–H bending patterns in Figures 5A and 5B supports our identifying the low-pH solution conformation as the inverted 1-ax/5-eq form. Through use of the 2067-cm^{-1} band of added SCN^- (0.1 M KSCN) as internal intensity standard, we derived relative molar scattering intensities of the C–H bending bands for each conformer. We thus were able to determine that, for the 0.10 M sodium IHP solutions examined, equal amounts of the two conformers coexist at pH 9.4 ± 0.1. This value coincides with the solution pH around which the change in appearance of the ^{13}C NMR spectra is centered (Figure 2), and also falls within

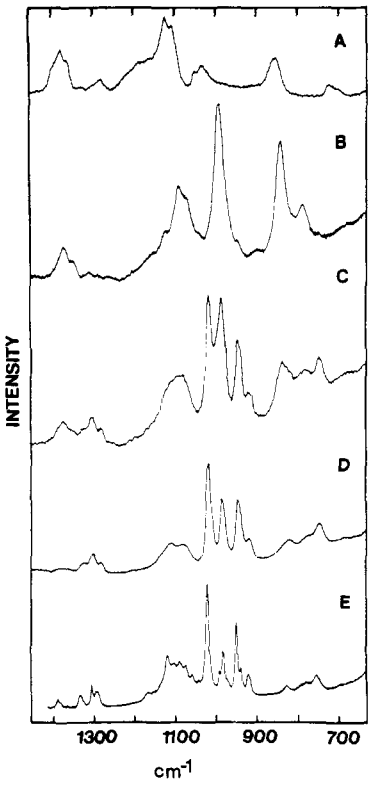

Figure 5. Partial Raman spectra of (A) a saturated solution of *myo*-inositol in D_2O; (B) 0.090 M sodium IHP in H_2O, pH 8.5; (C) 0.090 M sodium IHP in H_2O, pH 9.4; (D) 0.097 M sodium IHP in H_2O, pH 10.0; (E) the solid dodecasodium salt of IHP (as supplied by Sigma Chemical Co.). In spectrum A, there is some contribution at ca. 1200 cm^{-1} from the D_2O deformation vibration.

the range of the apparent pK_a values that we determined for the last three acid-dissociation equilibria of IHP (viz., 9.2–9.6).

All the available evidence therefore suggests that the ring inversion from 1-ax/5-eq to 5-ax/1-eq is triggered by one or more of these three acid-dissociation steps. The additional negative charge conferred on the phosphate oxygen atoms as the final protons are lost in solution evidently is best accommodated by the 5-ax/1-eq conformation, in which phosphate groups can be farther apart than in the 1-ax/5-eq form.[7]

The frequencies of bands in the C–H stretching region support our conformational structure assignments. The broad C–H stretching band for *myo*-inositol in H_2O (1-ax/5-eq hydroxyls) is centered at 2925 cm^{-1}, whereas the average frequency of the two most intense C–H stretching bands for solid $Na_{12}IHP$ (5-ax/1-eq phosphates) is higher, at 2958 cm^{-1}. Analogously, the low-pH IHP conformer in aqueous solution exhibits its C–H stretching band at ca. 2940 cm^{-1}, while the corresponding band for the high-pH form is found at ca. 2958 cm^{-1}.

Several experiments were performed to determine the effect of added alkali metal ions (viz., Li^+, Na^+, and Cs^+) on IHP's conformational equilibrium. In each case, an alkali metal chloride was added to a 0.10 M sodium IHP solution at pH 9.2 (pH arbitrarily chosen) so that the final solution was 1.0 M in added cation. The addition of each cation lowered the pH, which subsequently was readjusted to 9.2. The spectra of the resulting solutions indicated that each of the added alkali metal chlorides increases the proportion of high-pH conformer in solution by a moderate amount.[20] The extent of this effect is not equal for all three cations, however, and follows the order $Li^+ \sim Na^+ > Cs^+$. This suggests that, in addition to possible ionic-medium effects, one or more of these cations preferentially bind to and stabilize one of the IHP conformers. Two

possibilities exist: either Li^+ and Na^+ stabilize the high-pH form more than Cs^+ does, or Cs^+ stabilizes the low-pH form more than do Li^+ and Na^+. We favor the former alternative for the following reasons: (1) the 5-ax/1-eq conformer in solid $Na_{12}IHP$ appears to be stabilized by intramolecular Na^+ bridging of phosphate groups,[7] (2) the order of stability of alkali metal complexes with the related ligand, pyrophosphate, is $Li^+ > Na^+ > K^+$,[21] and (3) a new shoulder appears at ca. 1005 cm^{-1} in the spectrum of the IHP solution containing added Li^+, most likely the result of a PO_3^{2-} stretching vibration perturbed by Li^+ complexation. Since associations involving alkali metal ions are predominantly electrostatic, it is not surprising that the spectrum of the high-pH conformer is so little altered by the presence of these ions.

Knowing that alkali metal ions can stabilize the high-pH IHP conformation helps us to understand the discrepancy between the highest pK_a values we determined ($pK_a = 9.2$–9.6) and those determined previously by others through ^{31}P NMR measurements ($pK_a = 10$–12).[6] Instead of using Na^+ as the counterion, these earlier NMR workers used the large tetrabutylammonium ion, which we expect would bind to the 5-ax/1-eq anion much more weakly than does Na^+. Loss of this stabilizing influence would be accompanied by decreased acidity for the last three protons.

We were interested to find the Raman spectrum of solid (hydrated) Ca_6IHP to be characteristic of the conformer having 1-ax/5-eq phosphates (low-pH form): the pattern of its C–H bending bands is identical with that in Figure 5B,[20] its C–H stretching band is centered at ca. 2940 cm^{-1}, and it exhibits a skeletal stretching band at 845 cm^{-1} (cf. Figure 5B) rather than at ca. 750 cm^{-1} (cf. Figure 5D). In contrast to the spectrum of solid $Na_{12}IHP$ (Figure 5E), only one prominent band appears between 900 and 1150 cm^{-1} for the calcium salt; this band, at 1021 cm^{-1}, very likely represents appreciable contribution from symmetric PO_3^{2-} stretching motions.[22] The number and identity of the countercations in a solid IHP salt thus appear to be important in determining which conformer is preferred. Cation-dependent differences probably exist in the extent of intra- and intermolecular cation bridging of phosphate groups.

Acknowledgments. We are grateful to D. L. McKean, C. D. Sazavsky, and J. D. Wendel for help with the experimental part of this work.

Supplementary Material Available: Further discussion of the Raman spectra in Figure 5 and Raman spectra of *myo*-inositol, IHP with added alkali metal ions, and solid Ca_6IHP (5 pages). Ordering information is given on any current masthead page.

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