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Conformational Inversion Processes in Phytic Acid: NMR Spectroscopic and Molecular Modeling Studies

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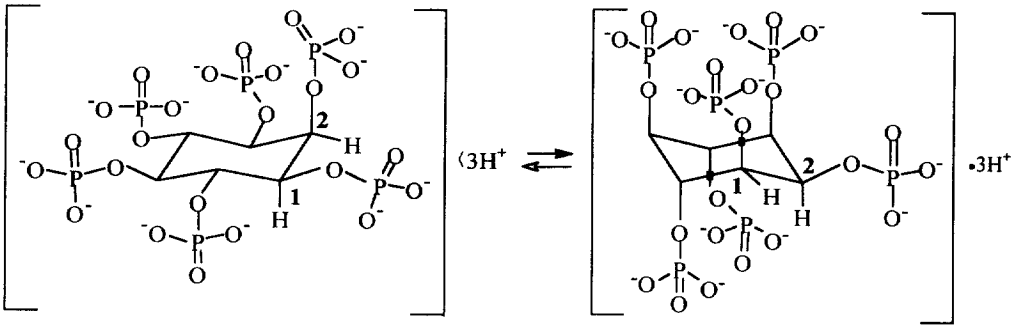
Abstract: NMR spectroscopy and computational studies show that phytic acid undergoes pH- and ion-dependent conformational inversion from the 1ax/5eq form to the 5ax/1eq form. The kinetics and energetics of the conformational inversion process are discussed.

Molecules that can be induced to undergo large changes in molecular shape rapidly and in a concerted manner are useful as molecular switches, sensors, transport carriers, and allosteric receptors. Some inositol phosphates, including *myo*-inositol hexakisphosphate (phytic acid, IP_6) undergo pH-dependent conformational inversion from the 1ax/5eq form to the 5ax/1eq form.[1,2] Below pH 9.0, phytic acid exists in the sterically-unhindered 1ax/5eq form and above pH 9.5 in the sterically-hindered 5ax/1eq form. Complete ionization of phytic acid to the dodecanionic form is necessary for conformational inversion to occur (the pKa of the three least acidic protons are between 9.0 and 9.5).[1] The inversion to the sterically-hindered 5ax/1eq form is believed to be due to complexation with metal ions which reduces electrostatic repulsion thereby stabilizing the sterically-hindered form.[1] To ascertain the energetics of the conformational switching process, we conducted experimental (NMR spectroscopy) and theoretical studies. Here we report on the effect of counterions on the conformational switching of phytic acid and on the kinetics and energetics of the conformational inversion process using dynamic NMR spectroscopy and molecular modeling.



1ax/5eq 5ax/1eq

Fig.1: The conformational interconversion of phytic acid between the 1ax/5eq and 5ax/1eq forms between pH 9.0 – 9.5.

(A) The pH-dependent NMR spectra showed dramatic broadening of NMR resonances between pH 9.0 and 9.5, thus suggesting that the 1ax/5eq and 5ax/1eq forms are in dynamic equilibrium (Fig. 1).[1] The presence of both conformations is revealed upon cooling the sample to about 5 °C, whereupon resonances characteristic of

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both conformations are evident. The rate of conformational inversion and the activation energy of the process were determined by dynamic NMR spectroscopy.[3] The temperature-dependent NMR spectra at pH 9.3 indicated that the coalescence temperature, T_c, for resonances H1/H3, H5, and H4/H6 was 15 °C.[4] Random delay exchange spectroscopy at 1.0 °C (Fig. 2) was carried out to assign resonances and to determine the difference in chemical shifts. The $\Delta\nu$ of protons 1 and 3, 4 and 6, 2, and 5 were determined, and the exchange rate and $\Delta G^{\#}$ were calculated using the Bloch equation and the Eyring equation.[3] The average $\Delta G^{\#}$ was 54.8 $\pm$ 0.8 kJ/mol. (B) The effect of alkali metal ions on the conformation adopted by phytic acid was investigated. Comparison of the spectra of phytic acid in the presence of different alkali metals at acidic pH (about 5.0), showed small changes in the chemical shifts of protons, but no significant changes in coupling constants were observed. This indicated that at pH 5.0, phytic acid adopts the 1ax/5eq form regardless of the counterion present. At pH 11 however, whereas the exclusive presence of the 5ax/1eq form was observed in the presence of Na^+, K^+, Rb^+, and Cs^+, in the presence of Li^+ ions the exchange-broadened spectrum was observed and this persisted up to pH 13.0. This indicates the inability of Li^+ ions to complex and thereby stabilize the 5ax/1eq conformation; this inability may be due to the larger hydrated radii of Li^+. To estimate the differences in the activation energy of the conformational inversion process, the T_c (at pH 9.1) was determined in the presence of different alkali metals. T_c, and therefore the activation energy, increased as follows: Rb^+ and Cs^+ (<0 °C), K^+ (10 °C), Na^+ (15 °C), and Li^+ (>55 °C). The trend in T_c paralleled the increase of hydrated radii, $Rb^+ \sim Cs^+$ (2.28 Å) > K^+ (2.32 Å) > Na^+ (2.76 Å) > Li^+ (3.40 Å). The results suggest that the ability of alkali metals to facilitate the conformational inversion of phytic acid decreases with the size of the hydrated ion. (C) When tetrabutyl ammonium hydroxide or tetramethyl ammonium hydroxide was used as the counterion, the 1ax/5eq conformer of phytic acid was the only one observed in the NMR spectrum up to pH 12; no NMR characteristics of the 5ax/1eq form were evident.

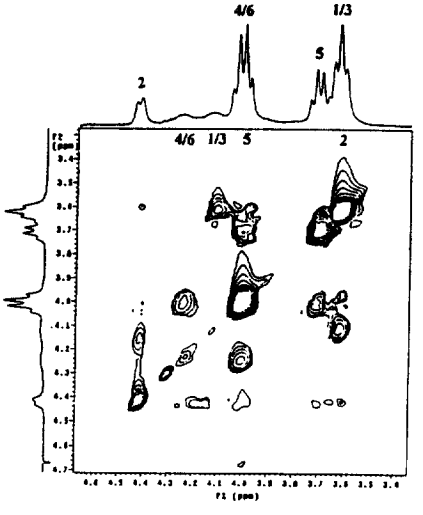

Fig. 2: Random delay exchange spectroscopy (EXSY)[5] of IP_6 at pH 9.2 with attached ^1H spectra. Protons of the 1ax/5eq conformer are indicated above the resonances and protons of the 5ax/1eq form, below.

Molecular modeling studies were completed for both the 1ax/5eq and 5ax/1eq conformations of phytic acid. Initially, Monte Carlo conformational searches were performed with the AMBER* force field using MacroModel[7], to determine the thermally accessible minimum energy conformations. Further conformational studies were then conducted with AM1, PM3, and DFT-B3LYP/6-31g(d) quantum mechanical methods and the AMBER* force field method, using the Gaussian 94[6] and MacroModel packages. The GB/SA method available in the MacroModel package was used to estimate the solvation energies. (A) For the fully protonated phytic acid, all methods predicted that, in vacuum and in aqueous environments, the 1ax/5eq conformation was more stable than the 5ax/1eq form (Table 1). This result is consistent with experimental data above. (B) In the dodecanionic form, all methods agreed with experimental results in predicting that the 5ax/1eq form was more stable than the 1ax/5eq form in vacuum. In aqueous solution, the PM3 and DFT methods predicted that the 5ax/1eq form was more stable (in agreement with experimental results), whereas AMBER* and AM1 predicted that the 1ax/5eq form was more stable (Table 1). (C) For the relative stabilities of the two conformations, the NMR results agree with the PM3 and DFT-B3LYP/6-31g(d)//PM3 methods in all cases. For the dodecanionic species in aqueous solutions the AMBER* and AM1 methods showed disagreement with experimental results, suggesting that they may not be as reliable methods for such highly charged species. (D) NMR coupling constants predicted by MacroModel for the most stable 1ax/5eq and 5ax/1eq (Fig. 3 & 4) conformations

Table 1:
Energy minimizations of *myo*-inositol hexakisphosphate conformers

Compound	In Vacuum (kJ/mol)				In Water (kJ/mol)		
	DFT w/ B3LYP / 6-31g(d)//PM3	AMBER*	PM3	AM1	AMBER*	AMBER*// PM3	AMBER*// AM1
Fully Protonated IP_6							
1ax/5eq	**-10745144.4**	**-995.5**	**-6269.7**	**-4083.8**	**-1169.7**	**-837.4**	**-827.9**
5ax/1eq	-10744978.3	-926.2	-6232.4	-3966.7	-1127.3	-760.9	-711.0
ΔE	(166.1)	(69.3)	(37.4)	(117.1)	(42.3)	(76.6)	(117.0)
Dodecanionic IP_6							
1ax/5eq	-10712527.9	12686.3	7820.1	9751.8	**-6531.5**	-4807.4	**-5238.4**
5ax/1eq	**-10712625.7**	**12456.1**	**7767.8**	**9678.2**	-6528.5	**-4981.4**	-5233.3
ΔE	(-97.8)	(-230.2)	(-52.3)	(-73.5)	(3.0)	(-174.0)	(5.1)
$\Delta E^{\dagger}$						(-117.0)	(59.2)

The numbers in bold indicate the conformer of lower energy.
ΔE Energy difference between 5ax/1eq and 1ax/5eq conformations ($\Delta E = E_{(5ax/1eq)} - E_{(1ax/5eq)}$)
$\Delta E^{\dagger}$ Energy difference between 5ax/1eq and 1ax/5eq conformations, with AMBER* solvation energies calculated at PM3 and AM1 optimized geometries

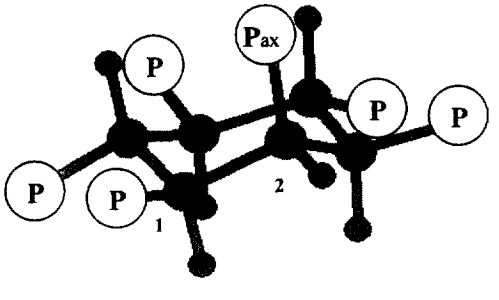

Fig. 4: PM3 Optimized 1ax/5eq *myo*-IP_6
(P=Phosphate groups)

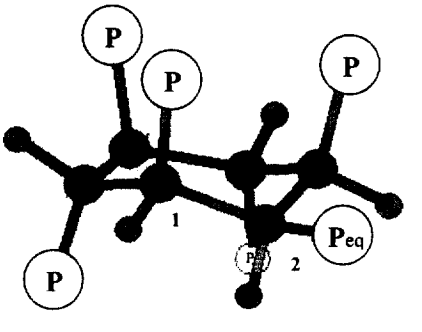

Fig. 5: PM3 Optimized 5ax/1eq *myo*-IP_6
(P=Phosphate groups)

compared well with experimental data. For the 1ax/5eq conformation (Fig. 3), $J_{1,2} = J_{2,3} = 2.0 \pm 0.2$ Hz, expt. 1.7 Hz, predicted; $J_{3,4} = J_{1,6} = 9.6 \pm 0.2$ Hz expt., 9.6 Hz predicted. For the 5ax/1eq conformation (Fig. 4), $J_{1,2} = J_{2,3} = 2.2 \pm 0.2$ Hz, expt., 2.4 Hz predicted; $J_{4,5} = J_{5,6} = 1.7 \pm 0.2$ Hz, expt., 0.7 Hz predicted. (E) The theoretical DFT predictions of large energy differences between the conformers at the pH extremes agree with the experimental results that there is no interconversion between the 1ax/5eq and 5ax/1eq conformers, except at intermediate pH of 9.0 – 9.5.

In summary, allosteric behavior of phytic acid is described. Changes in pH and counterions induce large changes in the molecular shape of the molecule. Molecular modeling predictions were consistent with NMR spectroscopic observations and provided information about the energies of the different conformers.

Acknowledgement

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4. This coalescence temperature, T_c, which is the lowest temperature at which the peaks coalesce into broad bands, was determined by monitoring the NMR spectra as a function of temperature.
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