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Conformational flexibility of inositol phosphates: influence of structural characteristics

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Abstract—Dynamic NMR and molecular modeling techniques were employed to investigate the influence of structural characteristics on the ring inversion processes of inositol phosphates. The results indicate that inositol phosphates that contain the *syn*-1,3,5-triaxial trisphosphate arrangement show great proclivity to adopt the sterically hindered conformation. These results suggest that the ability to form chelation cages with counter-ions is a major factor in the ring inversion processes of inositol phosphates.

Inositol phosphates play critical roles in signal transduction processes of living cells.[1] The conformational flexibility of biomolecules, including inositol phosphates, has a major impact on binding interactions with enzymes and receptors and thus on biological properties. We have previously reported on the conformational flexibility of inositol phosphates.[2,3] Chair–chair conformational interconversion of inositol phosphates is influenced by a number of factors including pH, the number and positions of phosphate groups on the inositol ring, and counter-ions. Whereas inositol hexakis- and pentakisphosphates show proclivity for chair–chair conformational interconversion, the presence of four or fewer phosphates on the inositol ring is insufficient to induce conversion to the sterically hindered 5ax/1eq form. Below pH 9.2, *myo*-inositol hexakisphosphate (phytic acid, Fig. 1, (**I**)) exists in the 1ax/5eq form and above pH 9.5 in the 5ax/1eq form. Between pH 9.2 and 9.5, when both 1ax/5eq and 5ax/1eq forms are at equilibrium, the coalescence temperature, T_c, is 15°C and $\Delta G^{\#}$ is 54.8±0.8 kJ/mol.[3] Complete ionization to the dodecanionic form is necessary for conformational inversion to the sterically hindered 5ax/1eq form; the stabilization of the 5ax/1eq form may be due to the ability of the *syn*-1,3,5-triaxial trisphosphate and *syn*-1,3-diaxial bisphosphate arrangements to form chelation cages with counter-ions. In this paper, we attempt to understand the influence of structural characteristics, including stereochemistry and substitution patterns, on conformational inversion. Towards this end, we have conducted NMR spectroscopic and molecular modeling studies of inositol hexakis- and pentakisphosphates.

1. *myo*-Inositol(1,3,4,5,6)P_5 (Fig. 1, (II))

(a) The chemical shift and coupling constant data from NMR spectra at various pH (2–13) were consistent with the presence of the 1ax/5eq conformation up to pH 10.5 and the 5ax/1eq form above pH 10.7. Between pH 10.5 and 10.7, significant broadening of the signals was observed and this suggested that the 1ax/5eq and 5ax/1eq forms were in dynamic equilibrium. (b) Temperature-dependent NMR spectra (3–40°C) at pH 10.7 indicated that the T_c was 37.5±2.5°C. Random delay exchange spectroscopy (EXSY) was conducted at pH 10.7 and 3°C to assign resonances in the two forms (Fig. 2). Exchange rate and $\Delta G^{\#}$ (59.6±0.5 kJ/mol) were calculated using the modified Bloch and Eyring equations.[5] (c) The value of $\Delta G^{\#}$ is significantly higher than that of phytic acid[2] (54.8±0.8 kJ/mol) even though both compounds contain a *syn*-1,3,5-triaxial trisphosphate arrangement on one face and a 1,3-diaxial bisphosphate arrangement on the other. This suggests that the phosphate group at C-2 does have an influence on the conformational inversion process even though it does not participate in the formation of the chelation cage. (d) To gain additional insight, molecular modeling studies were conducted as previously described.[3] Table 1 shows that DFT calculations in gas phase and

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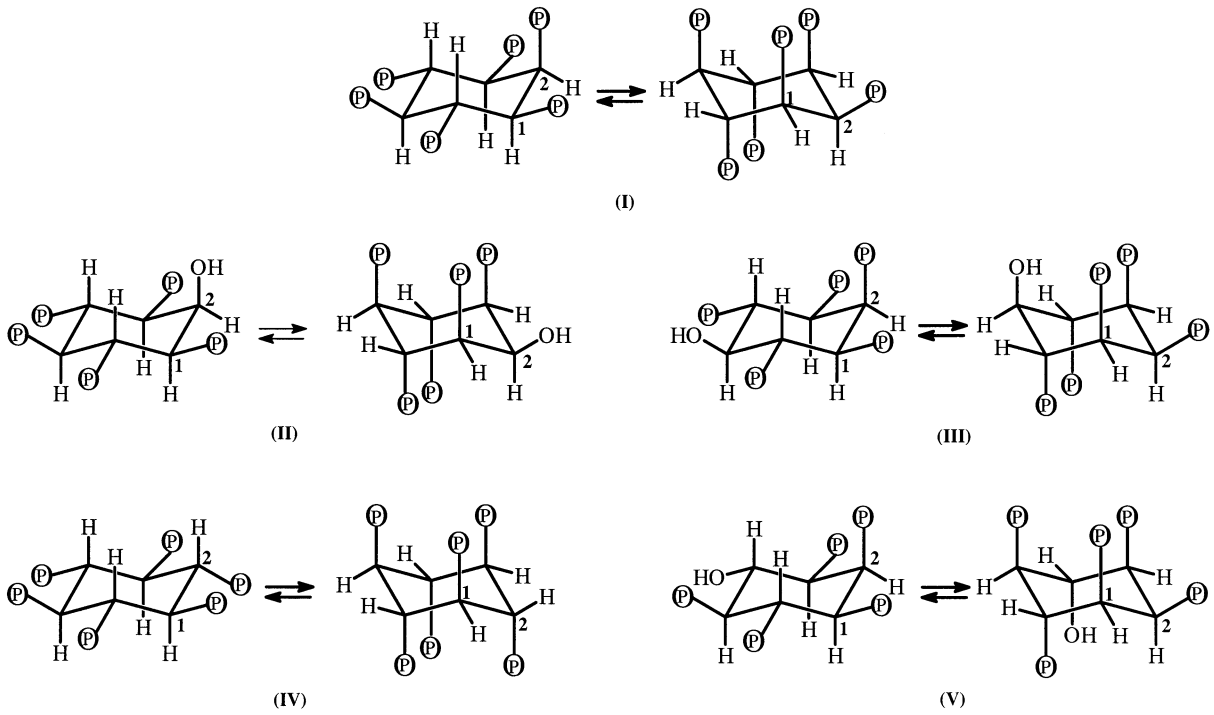

Figure 1. Conformational inversion of inositol phosphates.

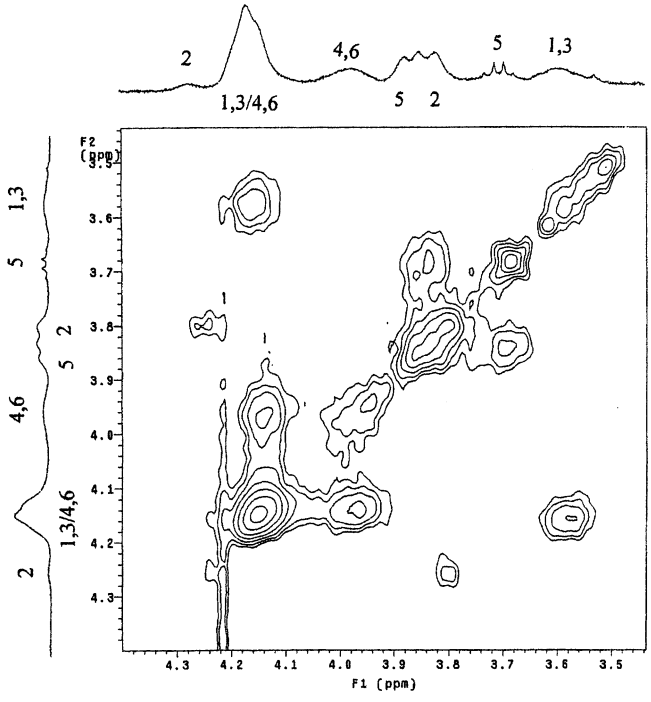

Figure 2. EXSY[4] of *myo*-inositol(1,3,4,5,6)P_5 at 3°C and pH 10.7. Protons of the 1ax/5eq conformer are indicated above the resonances and the 5ax/1eq form below.

aqueous solution are consistent with experimental results. The energy differences between the conformations are quite large thus excluding any equilibrium between the two forms.

2. *myo*-Inositol(1,2,3,4,6)P_5 (Fig. 1, (III))

(a) The pH-dependent spectra[2] indicates that the 1ax/5eq form is present up to pH 9.4 and above pH 9.5 both the 1ax/5eq and 5ax/1eq forms are in equilibrium; unlike the results of (**II**) above, the exclusive presence of the 5ax/1eq form was not observed. (b) Temperature-dependent NMR spectra (3–80°C) indicated that the coalescence temperature was 80°C and $\Delta G^{\#}$ was calculated to be 73.9±0.8 kJ/mol. (c) The relatively high value of $G^{\#}$ for (**III**) compared to (**I**) and (**II**) may be probably due to the lack of *syn*-1,3,5-triaxial trisphosphate arrangement on either face and, consequently, the inability to a form a tight chelation cage with counter-ions.

3. *scyllo*-Inositol-P_6 (Fig. 1, (IV))

The all *trans* isomer of inositol exhibited an unusually simple NMR spectrum (Fig. 3). (a) Up to pH 9, a broad doublet (J_{HP} about 5.3 Hz) was observed indicating that all protons are chemically equivalent (Fig. 3a); broadband phosphorus decoupling resulted in a sharp singlet (Fig. 3b). Above pH 9 (sodium conterion), a doublet (J_{HP}=11.0 Hz) was observed (Fig. 3d); broadband phosphorus decoupling resulted in a sharp singlet (Fig. 3e). Significant broadening of the NMR signal around pH 9.2 suggested exchange between the 6eq and 6ax forms. The H–C–O–P dihedral angles (calculated using a modified Karplus equation[6]) that correspond to J_{HP} of 5.3 (pH 5) are 43.2 or 109.3° and values that correspond to J_{HP} of 11.0 (pH 10) are 0.0 or 127.5°. To determine if one of the two dihedral angles was more

Table 1. Energy minimizations of *myo*-inositol(1,3,4,5,6)P_5 and *scyllo*-inositol-P_6 conformers

myo-Inositol(1,3,4,5,6)P_5	Gas phase (kJ/mol)	Aqueous (kJ/mol)	*scyllo*-Inositol-P_6	Gas phase (kJ/mol)	Aqueous (kJ/mol)
	DFT-B3LYP/6-31g(d)//PM3	AMBER*//PM3		DFT-B3LYP/6-31g(d)//PM3	AMBER*//PM3
	Fully protonated			Fully protonated	
1ax/5eq	**−9254853**	**−728**	6eq	**−10745117**	**−781**
5ax/1eq	−9254791	−658	6aq	−10745031	−736
ΔE	(62)	(70)	ΔE	(85)	(45)
$\Delta E^{\ddagger}$		(56)	$\Delta E^{\ddagger}$		(75)
	Decanionic			Dodecanionic	
5ax/1eq	**−9229833**	**−4535**	6ax	**−10712649**	**−5008**
1ax/5eq	−9229781	−4462	6eq	−10712484	−4724
ΔE	(−52)	(−73)	ΔE	(−165)	(−283)
$\Delta E^{\ddagger}$		(−70)	$\Delta E^{\ddagger}$		(−920)

The numbers in bold indicate the conformer of lower energy.
ΔE: Energy difference between 6ax or 5ax/1eq and 6eq or 1ax/5eq conformations ($\Delta E = E_{(6ax \text{ or } 5ax/1eq)} - E_{(6eq \text{ or } 1ax/5eq)}$).
$\Delta E^{\ddagger}$: Energy difference between 6ax or 5ax and 6eq or 5eq conformations; DFT//PM3 energies plus GB/SA solvation energies with the AMBER* force field.

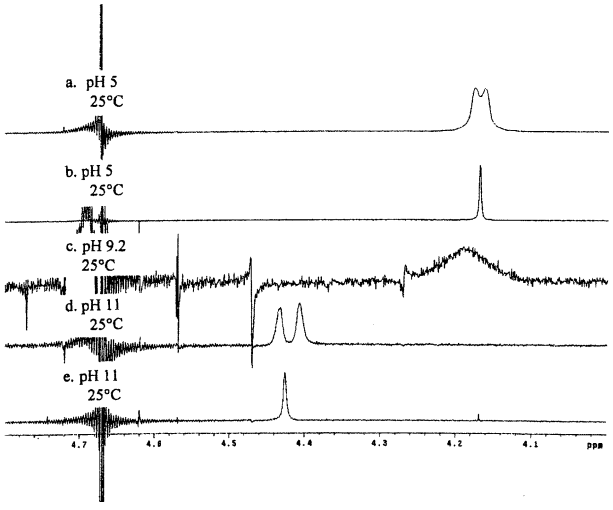

Figure 3. ^{1}H NMR spectra of *scyllo*-inositol-P_6 at different pH as indicated. Panels b and d are broadband phosphorus-decoupled ^{1}H spectra.

favorable, we separately imposed each of the angles on the optimized conformations. For the 6eq conformation visual inspection did not indicate any particular advantage between dihedral angles, 43.2 or 109.3°. For the 6ax, however, visual inspection did suggest that a dihedral angle of 127.5° formed a more favorable chelation structure than 0.0°. (b) To determine the T_c, temperature-dependent NMR spectra were recorded (below 0°C, H_2O/CH_3OH solution was employed). Distinct resonances corresponding to individual conformers were not observed at temperatures down to −20°C, thus indicating that the T_c was lower than −20°C and the $\Delta G^{\#}$ was less than 51.2±1.0 kJ/mol. (The spectra became very broad and noisy below −20°C.) (c) The low $\Delta G^{\#}$ observed for the conformational inversion of *scyllo*-IP_6 may be due to the presence of *syn*-1,3,5-triaxial trisphosphate arrangements on both faces of the cyclohexane ring in the 6ax form, thus facilitating the formation of chelation cages with counter-ions on both faces and stabilizing the sterically crowded form. (d) We were interested in the influence of the size of the counter-ion on the comparatively facile conformational inversion process of *scyllo*-inositol-P_6. When tetrabutyl ammonium ion was employed as the counter-ion, the broad doublet at pH 5 (Fig. 3a) became broader with increase in pH and remained so up to pH 12.5; however, the addition of NaCl at pH 12.5 transformed the spectrum to a well-resolved doublet (similar to Fig. 3e) with $J_{HP} = 11$ Hz. This confirmed that, as is the case with phytic acid, a metal counter ion of appropriate size (≤ 2.76 Å, hydrated radii), is indeed necessary for the conformational inversion process. (e) Table 1 shows that conformational predictions derived with the DFT method agree with experimental results for both gas phase and aqueous solution. The differences in the conformational energies in all cases are large enough to exclude any equilibrium between the two conformations at these levels of protonation. However, differences in energies calculated with the inclusion of solvation effects are unreasonably large and need to be viewed with caution for the dodecanionic conformation. Several repetitions in MacroModel yielded the same results.[7] Additional calculations using dipole and PCM SCRF techniques were attempted; however, because *scyllo*-IP_6 is symmetric and large the dipole method predicted negligible polarization energies and the PCM method incurred non-correctable errors.

Previous results have indicated that the T_c of *myo*-inositol (1,2,3,5,6)P_5[2] (Fig. 1, (V)), which contains the *syn*-1,3,5-triaxial trisphosphate arrangement on one face and lacks the 1,3-diaxial bisphosphate arrangement, is lower than that of (**IV**).[2] Thus, the value of $\Delta G^{\#}$ for the ring inversion process [(**IV**)<(**I**)<(**II**)<(**V**)<(**III**)], correlates well with the ability to form chelation cages with counter-ions. In summary, the stereochemistry and substitution patterns of phosphates have a significant influence

on the conformational inversion processes of inositol phosphates.

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