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Phosphate transfer from inositol pyrophosphates $InsP_5PP$ and $InsP_4(PP)_2$: A semi-empirical investigation

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Abstract—A novel phosphate transfer process involving the non-enzymatic transfer of a phosphate group from inositol pyrophosphates to serine residues in proteins has been recently reported. Semi-empirical calculations at the PM3/SM5.2 level were undertaken to explore the effect of inositol pyrophosphate structure and overall charge on the thermodynamics of this phosphate transfer.

Phosphorylation is a major component of cellular energy production and is essential for a diverse range of processes including the control of cellular pathways, hormone action and signal transduction.[1] Adenosine or guanosine triphosphates (ATP and GTP) are the most commonly used phosphate (Pi) donors in the cell; however a number of other biological compounds are capable of phosphate donation. Phosphoenolpyruvate and 1,3-bisphosphoglycerate, for example, are known to have transfer potentials greater than that of ATP,[1] and pyrophosphate (PPi) alone has been shown to fulfil the role of ATP in some reactions in certain parasitic protozoa.[2–5] In addition, inorganic polyphosphate (polyPi) has been shown to act as a phosphate donor through the polyphosphate kinase catalyzed conversion of polyPi and ADP to ATP.[6] The driving force for these reactions is the instability of the phosphoanhydride linkage caused by competing resonance and electrostatic effects as well as solvation effects.[1] Other biological compounds which contain similar linkages may prove to be potent phosphorylating agents.

Structurally complex inositol-based pyrophosphates have been identified in *Dictyostelium discoideum*,[7–9] yeast[10] and mammals.[11] These compounds can contain one (diphosphoinositol pentakisphosphate, $InsP_5PP$) or two (bis-diphosphoinositol tetrakisphosphate, $InsP_4(PP)_2$) pyrophosphate groups, with the exact placement varying with species; representative structures from *D. discoideum* and mammals[12] are shown in Figure 1. These inositol pyrophosphates have been shown to contribute to cellular chemotaxis,[13] apoptosis[14,15] and endocytosis,[10] and are rapidly turned over with their formation controlled by specific kinases[16–18] and phosphatases.[11] Recently, it has been discovered that $InsP_5PP$ can act as a *phosphorylating* agent in mammalian and yeast cellular extracts.[19] Snyder and colleagues have elegantly demonstrated that these inositol pyrophosphates can phosphorylate serine residues in proteins without enzyme participation.[19] The exact role that this process plays in these organisms is currently unknown; however, the ability of these compounds to phosphorylate cellular proteins may be critical.

PPi has been thoroughly studied on its own and as a model compound for ATP. These investigations have focused on its conformational and hydrolytic properties, as well as its ionization states, solvation and metal ion

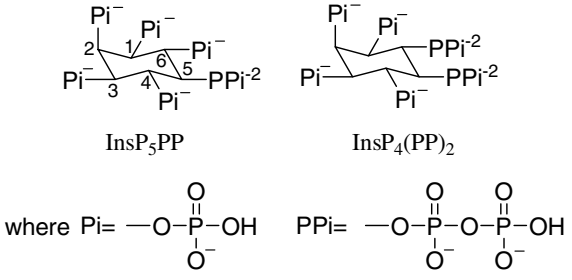

Figure 1. Structures of $InsP_5PP$ and $InsP_4(PP)_2$. A representative charge state for each molecule is indicated.

Keywords: Semi-empirical; PM3; SM5.2; Inositol pyrophosphate; IP_7; IP_8; $InsP_5PP$; $InsP_4(PP)_2$; Phosphate transfer; Phosphorylation.
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complexation and their effects on the structure and energetics of PPi.[20–25] These studies were performed using ab initio and density functional theory (DFT) methods in both the gas and aqueous phases.[26–30] In addition, methyl pyrophosphate has been studied using DFT methods.[30,31] Comparisons of theoretical and experimental results indicate that accurate modelling of Pi transfer from pyrophosphate moieties requires solvation consideration, as gas phase approaches do not well explain the experimental data:[20,21,24,29,32] the solvation energies of the substrates and products contribute substantially to the overall thermodynamic results. The thorough study of Pi donation by pyrophosphate groups is further complicated by the role that counter-ions are known to play in the thermodynamics of these reactions.[21,23–25,27,31] The proper choice and placement of the counter-ions adds another layer of complexity to the design of theoretical studies of these systems.

Initial studies of the pyrophosphate moieties of $InsP_5PP$ and $InsP_4(PP)_2$ performed using molecular mechanics techniques have indicated that these compounds may have a comparable or even greater energy of hydrolysis when compared to ATP.[7,8] Given the importance that this new role for $InsP_5PP$ and $InsP_4(PP)_2$ may play in cellular physiology,[19] a more detailed study of the thermodynamics of these compounds appears warranted. Due to the large number of ionizable groups present in these molecules, multiple ionization and tautomeric states must be examined, which makes the speed of the calculations to be performed an important factor in the modelling method selected. Initially, DFT studies at the 6-31++G** level were performed (data not shown); however, these calculations were very lengthy, even when parallelized, and the large number of negative charges present in the $InsP_4(PP)_2$ structures frequently resulted in a lack of convergence; this approach was therefore not further pursued. As an alternative, semi-empirical methods were chosen as they were more rapid than higher-level ab initio or DFT approaches.[33] While semi-empirical techniques can give artificially large activation enthalpies, the relative magnitudes of various effects are often realistic[34] as these elevated effects frequently cancel during the determination of reaction pathway enthalpies. Previous work studying the conformations of phytic acid, ($InsP_6$), has shown that the semi-empirical PM3 method and DFT methods can accurately predict the conformation of $InsP_6$,[35] despite the limitations of semi-empirical techniques.[36,37] In contrast, molecular mechanics methods using the AMBER* force field and the semi-empirical AM1 method did not predict the correct conformation.[35] The PM3 method has been used extensively to successfully model phosphorus[38,39] and phosphate groups.[40–42]

All calculations utilized the PM3 method[43,44] with the SM5.2 implicit water solvation model as implemented in AMPAC (version 8.16).[43–48] When a structure was found to have an H_f value which varied dramatically from other conformers of similar charge, a dihedral angle drive was performed on the pyrophosphate group to overcome energy barriers encountered during minimization. In almost all cases, this resulted in an H_f value that was in better agreement with other similar structures. A complete description of the computational methods used can be found in the Supplementary Information. Due to the inherent complexity of the various protonation and tautomeric states of the molecule, these calculations were not further complicated by the inclusion of counter-ions: the difficulty in predicting the starting positions of such counter-ions (such as Ca^{2+}, Mg^{2+}), as well as consideration of their force field parameterization, would have added a prohibitive layer of complexity to this preliminary study. $InsP_6$ has been shown to complex cations in solution;[49,50] therefore it is important to be aware that the presence of counter-ions will make a contribution to the overall energetics of the system. For example, variations in the concentration of Mg^{2+} can cause changes of up to ~1 kcal/mol in the ΔG^{o}_{obs} of the hydrolysis of ATP.[51] The focus of this preliminary work was to determine the effect of increasing charge on ΔH_f for phosphorylation by inositol pyrophosphates and to compare the ΔH_f values for $InsP_5PP$ and $InsP_4(PP)_2$ to each other and to PPi and methyl pyrophosphate (MeOPPi) as model systems.

The reported biological phosphorylations by $InsP_5PP$ have been localized to serine residues,[19] therefore, methanol was used as the model for this amino acid side chain. Initial calculations focused on Pi transfer from PPi, MeOPPi, cyclohexyl-PPi and *myo*-inositol-5-pyrophosphate (InsPPi) utilizing multiple protonation states of the pyrophosphate-containing compounds. InsPPi was used as a model for the inositol framework omitting the steric or electronic contributions from the multiple phosphate moieties present in $InsP_5PP$ and $InsP_4(PP)_2$. PPi has pK_a values of 0.85, 1.96, 6.68 and 9.39 and therefore will likely have a charge of −2 or −3 at physiological pH, while the product of methanol phosphorylation, MeOPi, has pK_a values of approximately 1.1 and 6.36 and therefore will likely have a charge of −1 or −2 at physiological pH.[52,53] The ΔH_f values found by these preliminary calculations are shown in Table 1. These results indicate that when comparing different ionization states that yield the same product, Pi donation may be more favourable when the starting structure has a higher charge, for example donation by PPi(−3) to form MeOPi(−1) is predicted to be favoured over PPi(−2) reactions. In addition, the formation of MeOPi(−2) is predicted to be more favourable than the formation of MeOPi(−1) when the same starting ionization states are involved. These results indicate that the overall thermodynamics of these reactions will be determined by the ionization states of both the reactants and products of these reactions; therefore, the present study included as many ionization and tautomeric states for $InsP_6$, $InsP_5PP$ and $InsP_4(PP)_2$ as necessary.

Previous studies have reported the X-ray structure of $InsP_6$[54] and the NMR determination of the pK_a values for each of its phosphate moieties (Table 2).[55] NMR data have shown that in the completely protonated form (charge = 0), $InsP_6$ exists in the 5 axial/1 equatorial (5a/1e) conformation; however, when the charge = −6, the lowest energy conformation is that of a 5e/1a conformation.[35,56,57] This has also been previously predicted by

Table 1. ΔH_{rxn} values computed using PM3/SM5.2 for the cleavage of various pyrophosphates by methanol to yield methylphosphate

Pi[a] Donor	→	Product	ΔH_{rxn}[c]	Pi Donor	→	Product	ΔH_{rxn}
Forming MeOPi(−1)[b]				Forming MeOPi(−2)			
PPi(−2)[d]	→	Pi(−1)	−8.9	PPi(−3)	→	Pi(−1)	−13.9
PPi(−3)	→	Pi(−2)	−12.9				
MeOPPi(−2)	→	MeOPi(−1)	−7.2	MeOPi(−3)	→	MeOPi(−1)	−15.2
Cyclohex-PPi(−2)[e]	→	Cyclohex-Pi(−1)	−7.3	Cyclohex-PPi(−2)	→	Cyclohex-Pi(−1)	−14.8
Cyclohex-PPi(−3)	→	Cyclohex-Pi(−2)	−14.3				
InsPPi(−2)[f]	→	InsPi(−1)	−2.0	InsPPi(−3)	→	InsPi(−1)	−6.2
InsPPi(−3)	→	InsPi(−2)	−14.8				

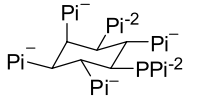
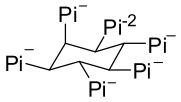
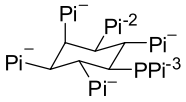
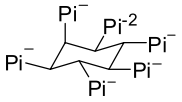
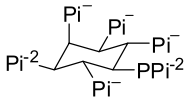
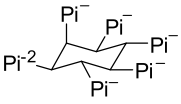
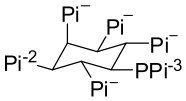
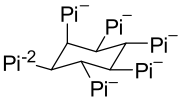
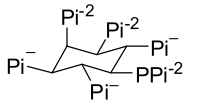
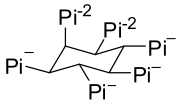
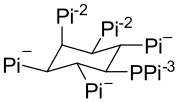
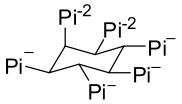
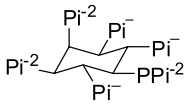
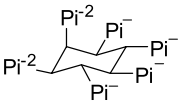
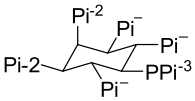
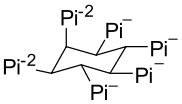
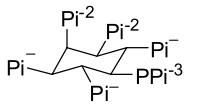
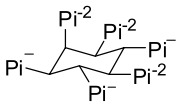
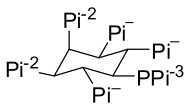
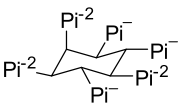
[a] Pi, phosphate.
[b] MeOPi, methyl phosphate.
[c] In kcal/mol.
[d] PPi, pyrophosphate.
[e] Cyclohex, cyclohexyl.
[f] Ins, inositol.

Table 2. pK_a values of phytic acid ($InsP_6$)[55]

pK_a	Carbon number			
	C-1/C-3	C-2	C-4/C-6	C-5
pK_a1	1.5	1.1	2.1	1.7
pK_a2	5.7/12.0	6.9	10	7.6

PM3 calculations in the gas phase, as well as DFT calculations in the gas and aqueous phase.[35] The pK_a values or conformational preferences for $InsP_5PP$ and $InsP_4(PP)_2$ however have not yet been theoretically or experimentally determined.

Table 3. ΔH_{rxn} values computed using PM3/SM5.2 for the cleavage of certain $InsP_5PPs$ by methanol to yield methylphosphate

$InsP_5PP$	→	$InsP_6$	ΔH_{rxn}[a]	$InsP_5PP$	→	$InsP_6$	ΔH_{rxn}[a]
Forming MeOPi(-1)				Forming MeOPi(-2)			
pH 5.7				**pH 5.7**			
	→		-15.7		→		-32.1
	→		-14.9		→		-31.8
pH 6.8				**pH 6.8**			
	→		-24.9		→		-38.3
	→		-15.9		→		-33.9
pH 7.6				**pH 7.6**			
	→		-22.6				ND[b]
	→		-19.9				ND

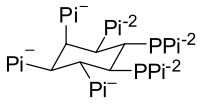
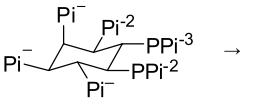
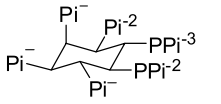
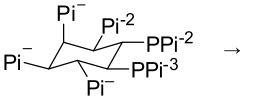
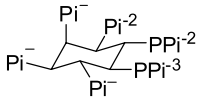
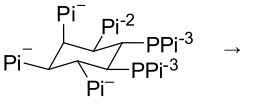
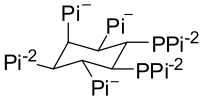
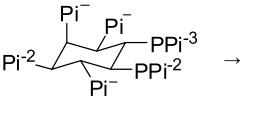
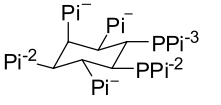
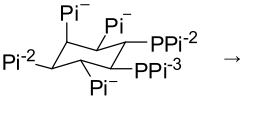
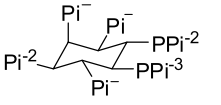
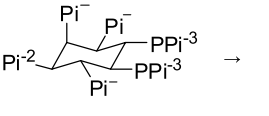
[a] In kcal/mol. [b] Not determined as further deprotonation is improbable due to the increasing negative charge on $InsP_6$.

Geometry optimizations were performed on $InsP_5PP$ and $InsP_4(PP)_2$ using the $InsP_6$ protonation pattern as a starting point with a 5e/1a starting conformation. A variety of protonation states which differed from the $InsP_6$ scheme were also studied as the charge distribution of $InsP_5PP$ or $InsP_4(PP)_2$ could differ from that of IP_6 due to the presence of the PPi groups. The products, MeOPi and $InsP_6$, were also evaluated with various protonation states. Select calculations producing protonation states of $InsP_6$ consistent with those found by NMR at pH values of 5.7–7.6 are shown in Table 3. A more encompassing set of calculations for Ins_5PP are presented in the Supplementary Information.

The transfer of Pi from $InsP_5PP$ to methanol is computed to be thermodynamically favourable in all cases. Similar to the results for PPi alone, the formation of MeOPi(−2) is predicted to be favoured over MeOPi(−1). In general, increasing the negative charge around the inositol ring also increases the predicted favourability of Pi group donation. This is likely due to increased electrostatic repulsion on the ring. The data presented in Table 3 indicate that phosphate donation by $InsP_5PP$ is predicted to be thermodynamically favoured over donation by PPi. There are subtle variations between the predicted ΔH_f values for tautomers of the same charge; however, this may be due to the alteration of conformation caused by changes in the placement of the charges around the ring, as well as changes in internal hydrogen bonding.

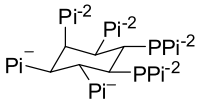
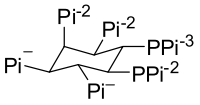
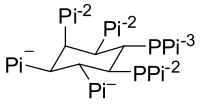
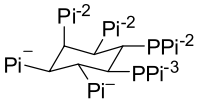
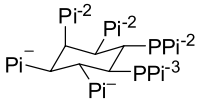
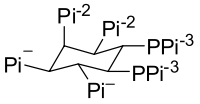
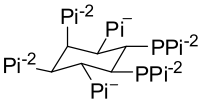
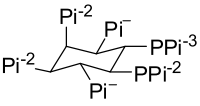
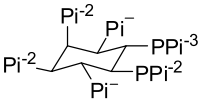
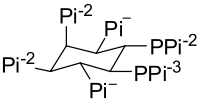
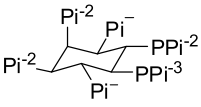
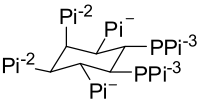
Calculations for phosphate donation by $InsP_4(PP)_2$ which produces $InsP_5PP$ at various protonation states consistent with the known pK_a values for $InsP_6$ are shown in Tables 4 and 5. Consistent with the PPi and Ins_5PP results, the formation of MeOPi(−2) is predicted to be favoured over MeOPi(−1) for the same starting tautomers. Similar to Ins_5PP, an increase in the charge on the inositol generally increases the predicted favourability of phosphate donation. A comparison of the values in Tables 3–5 indicate that when comparing structures at the same pH, forming the same MeOPi, $InsP_4(PP)_2$ is predicted to be a thermodynamically better phosphate donor than $InsP_5PP$. Steric and electrostatic repulsions caused by the adjacent pyrophosphate and phosphate groups are likely the cause of the predicted increased favourability. Similar to $InsP_5PP$, there are variations between the H_f values of tautomers of the same charge; as mentioned earlier, this may be due to conformational variations and changes in internal hydrogen bonding. Further analysis is warranted to determine the thermodynamic contribution of each phosphate on the inositol ring to the overall thermodynamics of each Ins_5PP and $InsP_4(PP)_2$ structure. In addition, the inclusion of at least one solvation sphere of explicitly defined water molecules should be a future approach.

Table 4. ΔH_{rxn} values computed using PM3/SM5.2 for the cleavage of certain $InsP_4(PP)_2$s by methanol to yield methylphosphate at pH 5.7

$InsP_4(PP)_2$ → $InsP_5PP$ Forming MeOPi(-1)	ΔH_{rxn}[a]	$InsP_4(PP)_2$ → $InsP_5PP$ Forming MeOPi(-2)	ΔH_{rxn}
	-29.7		-35.0
	-18.6		-35.8
	-19.5		-52.9
	-17.9		-35.7
	-18.8		-35.7
	-17.6		-53.2

[a]In kcal/mol.

Table 5. ΔH_{rxn} values computed using PM3/SM5.2 for the cleavage of certain $InsP_4(PP)_2$s by methanol to yield methylphosphate at pH 6.8–7.6

$InsP_4(PP)_2$ → $InsP_5PP$ Forming MeOPi(-1)	ΔH_{rxn}[a]	$InsP_4(PP)_2$ → $InsP_5PP$ Forming MeOPi(-2)	ΔH_{rxn}
	-30.6		-39.6
	-14.9		-46.6
	-33.2		-53.8
	-28.8		-48.5
	-30.6		-48.0
	-30.1		-50.8

[a]In kcal/mol.

It is clear from this level of study that increasing the charge of a molecule and steric bulk near the pyrophosphate group generally tends to increase the predicted thermodynamic favourability of Pi transfer. The highly charged and sterically congested structure of $InsP_4(PP)_2$ in an overall charged state of −8 (as seen in Fig. 1) can be appreciated in Figure 2 where the electrostatic potential is mapped onto the electron density of this compound.

Preliminary calculations on ATP, ADP and model compounds triphosphate, and methyl triphosphate were also performed.[58] These results indicate that the transfer of a phosphate group from $InsP_5PP$ and $InsP_4(PP)_2$ to methanol is as favourable, and in some cases more so, than transfer from ATP, and ADP. These results are preliminary and further studies must be performed to confirm these results due to the limitations of the current approach.

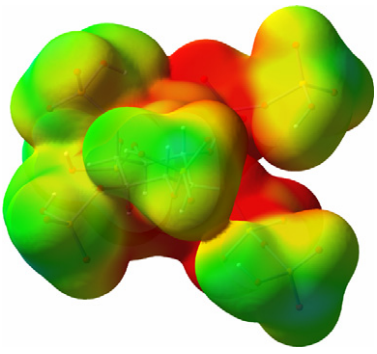


Figure 2. Electrostatic potential (using a range of −0.8 (red) to −0.5 (blue) kcal/mol) mapped onto the electron density isosurface (0.0004 electrons/au^3) for $InsP_4(PP)_2$ with a charge of −8 (as in Fig. 1).

Although a specific value for the transfer potential for these inositol pyrophosphates cannot be easily determined due to the expected ensemble of charged states that would exist for these compounds, it is clear that biological systems can control Pi transfer potential from pyrophosphates by tuning the steric and electrostatic environments of the pyrophosphate moiety. This information should contribute to our understanding of this novel cellular phosphorylation process.

Acknowledgments

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Supplementary data

Additional information including a complete listing of the thermodynamics of the various protonation states

and their reactions can be found in the Supplementary Information. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.09.066.

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58. Preliminary calculations performed on ATP and ADP yield enthalpies in the range of −2.2 to −11.8 kcal/mol for the donation of a phosphate group to methanol. Please see the Supplementary Information for more details.