Selective Hydrogen Bonding as a Mechanism for Differentiation of Sulfate and Phosphate at Biomolecular Receptor Sites

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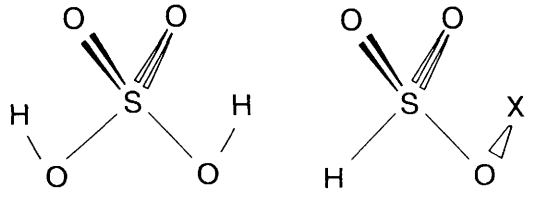
Ab initio calculations on protonated sulfuryl and phosphoryl species demonstrate and quantify a stereochemical differentiation of hydrogen bonding to sulfate and phosphate.

Sulfate biomolecules, although far less studied than their phosphate counterparts, are being revealed to play an increasingly important role *in vivo*. In sulfated glycosaminoglycans this role is in maintaining structure and function.[1] Indeed, in heparin, a defined regio- and stereo-chemistry of the oligosaccharide sulfate groups is essential for biological activity. Replacement of one sulfate group by phosphate in the essential pentasaccharide of heparin leads to loss of biological activity.[2] Selectivity in binding is further illustrated in prokaryotic, periplasmic phosphate[3a] and sulfate[3b] binding proteins, which demonstrate $>10^5$ selectivity for binding phosphate (mono- or di-anion) over sulfate and sulfate over phosphate, respectively. In both proteins a large hydrogen bonding network rather than electrostatic interactions accounts for the high affinity. Recent crystal structure determination of triosephosphate isomerase shows an altered hydrogen bonding network when sulfate replaces phosphate at the active site.[4]

How are binding sites able selectively to bind sulfate over phosphate? We have performed *ab initio* calculations including full geometry optimization of $H_2PO_4^-$, HPO_4^{2-}, H_3PO_4, H_2SO_4, HSO_4^-, H_2SO_3, $MeHSO_3$ using the HONDO-8 program.[5] These calculations: (*i*) demonstrate a marked difference in the stereochemistry of bonding of hydrogen to sulfate and to phosphate; and moreover (*ii*) point to the possibility of a stereochemically defined hydrogen bonding network able to differentiate between sulfate and phosphate at biomolecular receptor sites.

The conformational preference of the H–O bonds of $H_nSO_4^{(n-2)}$ and $H_nPO_4^{(n-3)}$ is an indicator of the preferred stereochemistry for hydrogen bonding to sulfate and phosphate. Comparison of the preferred torsional angle about the P–OH and S–OH bonds for all optimized structures shows a marked difference between sulfuryl and phosphoryl species (Fig. 1). Sulfuryl species strongly favour synperiplanar (sp) conformations with respect to the S=O bond.† Phosphoryl species show a less marked preference for synclinal (sc) conformers with respect to the P=O bond.† However, it has been stated that the conformational energy surface of phosphoryl species is dominated by dipole–dipole interactions,



I **II** X = Me, τ (COS=O) = 8.0°
X = H, τ (HOS=O) = 10.0°

† With reference to: formal double bonds for a, c, e, g, h, i, j (Fig. 1); partial double bonds b, f, k (Fig. 1); and P–OH bond of d (Fig. 1).

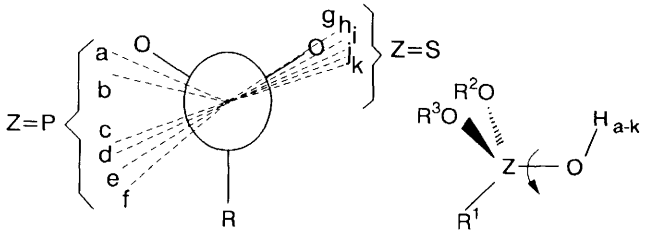


Fig. 1 Newman projection along (H)O–Z bond showing correlation of P–OH and S–OH torsion angles, relative to R^3O and R^2O respectively, for stable conformers[a]

Label	Compound	Torsion angle	R^1	R^2	R^3	Comment
(a)	H_3PO_4	−8°	OH	H	—	Local energy minimum [$E = E$(global) + 0.325 kcal mol^{-1}]
(b)	$H_2PO_4^-$	−24°	OH	δ−	δ−	Global energy minimum
(c)	H_3PO_4	−42°	OH	H	—	Global energy minimum
(d)	$H_4PO_4^+$	−45°	OH	H	H	Energy minimum from torsional energy scan
(e)	H_3PO_4	−60°	OH	—	H	Local energy minimum, second OH
(f)	HPO_4^{2-}	−66°	Oδ−	δ−	δ−	Global energy minimum
(g)	$H_3SO_4^+$	0°	OH	H	—	Energy minimum from torsional scan
(h)	H_2SO_4	2°	OH		—	Local energy minimum [$E = E$(global) + 2.31 kcal mol^{-1}]
(i)	H_2SO_3	10°	H		—	Global energy minimum[b]
(j)	H_2SO_4	13°	OH		—	Global energy minimum
(k)	HSO_4^-	25°	δ−	δ−	δ−	Global energy minimum

[a] Global and local energy minima are identified by potential energy surface scans of the (H)O–Z torsional surface and full optimization of the minimum energy structures thus located. 1 cal = 4.184 J. [b] Local minima at −12 °C for both H_2SO_3 and $MeHSO_3$ are within 0.1 kcal mol^{-1} of global minima.

with a secondary contribution from internal hydrogen bonding.[6] Since these effects should be greatly attenuated at a receptor site, it is essential to determine their influence on conformation, before extrapolation of the results to interactions at biomolecular receptor sites.

The dependence of energy and overall dipole on rotation about the P–OH and S–OH bonds was examined for various conformers of H_2SO_4, $H_4PO_4^+$ and $H_3SO_4^+$. In all cases, no obvious correlation exists between overall dipole and conformational energy.‡ For example, in H_2SO_4 with one O–H bond fixed synclinal with respect to the S=O bonds, the conformer of maximum energy (**I**) has the minimum overall dipole. In addition, steric factors and intramolecular hydrogen bonding are contraindicated as dominant influences on conformational energy. Fully optimized structures for sulfonic acid and its methyl ester both retain sp conformations (**II**), despite the absence of internal hydrogen bonding and the presence of steric repulsion in the methyl ester. One must conclude, contrary to Ewig and Van Wazer,[6] that dipole and internal hydrogen bonding effects do not dominate the conformational energy of these phosphoryl and sulfuryl species.§

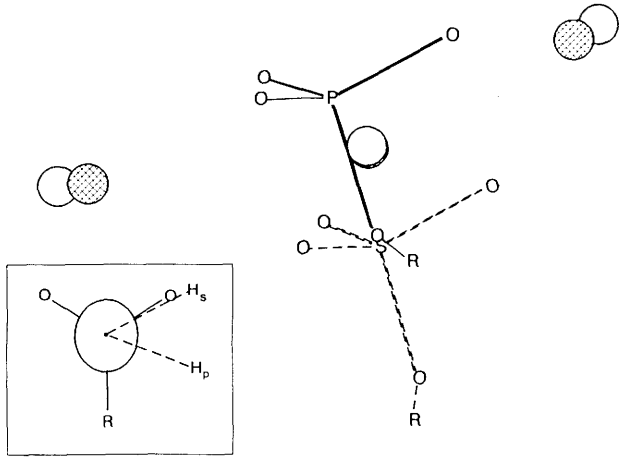


Fig. 2 Inset: Newman projection as Fig. 1 showing preferred sp (H_S, sulfate) and sc (H_P, phosphate) hydrogen bonds. Main: Three hydrogen bond donor heavy atoms positioned 2.8 Å from oxygen acceptors: solid spheres sc to HPO_4^{2-} (R = H) and hashed spheres sp to HSO_4^- (R = H). The hydrogen bond donors have been superimposed by least-squares fitting causing considerable translocation of sulfate relative to phosphate.

Comparison of the calculated conformational preferences presented herein (Fig. 1) with crystal structure data on hydrogen bonding to biomolecules containing the RPO_3^{2-} and RSO_3^- moieties[8] demonstrates a striking similarity. The sp preference for sulfate is further corroborated by calculations on $HSO_4^- \cdots H_3O^+$ which forms a strong hydrogen bond 2–3 kcal mol^{-1} more stable in the sp than the sc conformer.¶

‡ Torsional energy profiles are given by:

$$E = V_0 + V_1 \cos(\theta) + V_2 \cos(2\theta) + V_3 \cos(3\theta),$$

where for sulfuric acid: $V_0 = -694.64323$ au, $V_1 = 0.001247$, $V_2 = 0.002725$, $V_3 = 0$, $\theta = \tau$[H–O–S–O(H)], and for $H_4PO_4^+$; $V_0 = -638.76629$ au, $V_1 = 7 \times 10^{-7}$, $V_2 = 6 \times 10^{-7}$, $V_3 = 1 \times 10^{-3}$, $\theta = \tau$(HOPO) + 15.346°.

§ It remains to conclude that the dominant influence is stereoelectronic in origin. The proposed anomeric effect at phosphorus has been discussed with reference to orbital mixing of sp^3 lone pairs on oxygen with adjacent σ* (P–O) orbitals (see ref. 7*a* for a full critique), although its proponents have recently reconsidered its basis.[7b] According to the dogma of the anomeric effect the conformational preferences observed in this work are described by dominant $n(sp^2) \rightarrow \sigma^*$ orbital mixing in sulfate[7c] and $n(sp^3) \rightarrow \sigma^*$ in phosphate, although this description is unlikely to reflect the true molecular orbital basis for the observed stereoelectronic effects.

¶ Geometry optimized, linear, non-bifurcated[9a,b] hydrogen bond. Optimal treatment of hydrogen bonding requires a level of calculation untenable for these large systems.[9c]

The energy required to rotate: (*a*) one OH of H_2SO_4 from sp to sc is 2 kcal mol^{-1}; and (*b*) one OH of $H_4PO_4^+$ from sc to sp is 0.5–1.0 kcal mol^{-1}.‖ The cumulative effect of many stereochemically defined hydrogen bonds at a biomolecular receptor site is therefore presented as a mechanism for differentiation of sulfate and phosphate (Fig. 2). Moreover these resuls allow parameterization of molecular mechanics force fields to extend study to interaction with protein binding sites themselves.

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‖ The selectivity of periplasmic sulfate binding protein is shown by the difference in binding energy: $\Delta G(SO_4^{2-}) - \Delta G(HPO_4^{2-}) > 7.7$ kcal mol^{-1} [which is unlikely due to the extra hydrogen, since $\Delta G(CrO_4^{2-}) - \Delta G(HCrO_4^-) = 1.3$ kcal mol^{-1}].[3c] However, the nonadditivity of hydrogen bond strengths and the potential for relaxation of the binding site amino acid residues in the presence of an unnatural substrate must be considered in any comparison with the differential conformational energies calculated in this work.

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