Stereochemistry of Phosphate–Lewis Acid Interactions: Implications for Nucleic Acid Structure and Recognition

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Abstract: The geometry of phosphate–Lewis acid interactions has been examined by using the Cambridge Structural Database. Interactions involving metal ions display preferentially syn, unidentate, and out-of-plane coordination stereochemistry with regard to the phosphinyl ($-PO_2^-$) group. In contrast, hydrogen bond donors display a very slight preference for anti stereochemistry with the phosphinyl group, and these interactions do not tend toward in-plane interaction with the phosphinyl group. In general, preferential phosphate–Lewis acid geometry contrasts markedly with carboxylate–Lewis acid geometry, and our results are useful in the interpretation of nucleic acid structures and protein–nucleic acid interactions observed by macromolecular X-ray crystallography. Another application of our results concerns the use of phosphinyl derivatives as transition-state analogues; the electronic distribution of the phosphinyl moiety approximates that of the proteolytic transition state, but we present an upper limit to this approximation.

The anionic phosphinyl portion ($-PO_2^-$) of the phosphate group, as it comprises the backbone of nucleic acids, may interact with Lewis acids such as metal ions or hydrogen bond donors. Although the preferred geometry of such interactions has not been detailed, this geometry must nevertheless play a role in protein–nucleic acid recognition mechanisms.[1] If the planar phosphinyl group is an analogue of the anionic carboxylate group, then perhaps these groups share a common preferential stereochemistry as each interacts with Lewis acids. Specifically, the stereochemistry of carboxylate–Lewis acid interaction is defined as syn or anti (Figure 1).[2] The carboxylate group typically prefers a syn-oriented interaction with a metal ion,[3] a hydrogen bond donor,[2] or a covalently bound proton.[4] Additionally, the interaction preferably occurs in the plane of the carboxylate, presumably in order to complex the oxygen sp^2 lone electron pair. We now extend the syn/anti nomenclature to describe the interactions of the phosphate monoanion with Lewis acids such as metal ions or hydrogen bond donors (Figure 1). Do phosphates, like carboxylates, prefer in-plane and syn coordination geometry with Lewis acids?

In order to answer this question, we have analyzed the stereochemistry of phosphinyl–metal ion and phosphinyl–hydrogen bond donor interactions as extracted from the Cambridge Structural Database (CSD).[5] Statistical analysis of independent intermolecular interactions, as extracted from this massive database, has led to the characterization of the preferential stereochemistry corresponding to various structural motifs.[6] These stereochemical statistics are especially useful toward the interpretation of macromolecular crystallographic structures, because such structures are typically less well determined than those of small molecules. Unexpectedly, the results of our investigation suggest that the preferential stereochemistry of phosphate–Lewis acid interactions differs from that of carboxylate–Lewis acid interactions.

Our results provide a foundation for the functional interpretation of nucleic acid structures where phosphinyl–metal ion interactions are prevalent. For example, it is known that magnesium binds to loops and bends of the tRNA backbone and stabilizes the three-dimensional structure of the tRNA molecule.[7] An understanding of these interactions is also important in attempts to probe the tertiary structure of the catalytic RNA molecule and its interaction with metal ions.[8]

Phosphinyl–hydrogen bond donor interactions are equally important to the understanding of nucleic acid structure and function. For example, the three-dimensional structures of several repressor–operator complexes are replete with hydrogen bond contacts between the protein and the phosphate backbone (as well as the bases) of the DNA operator sequence.[1b,c,9] Interestingly, the *trp* repressor–operator complex shows no direct hydrogen bonds with bases known to contribute to the specificity of repressor–operator association: Sigler[1b] has proposed that the 28 hydrogen bonds between the repressor protein and operator phosphates support the hypothesis of indirect readout. In short, this hypothesis requires that the local base-pair sequence of the operator confers a unique conformational deformation upon genomic B-DNA: this unique conformation allows for specific recognition by, and tight binding with, the repressor protein. Direct interaction of the protein with specific bases is not an exclusive requirement for recognition. If indirect readout, or likely some combination of both direct and indirect modes of recognition, is important for understanding the structural features of protein–nucleic acid affinity, then a nucleic acid binding protein should have a stereochemically precise arrangement of hydrogen bond donors within its binding groove. These hydrogen bond donors should be poised, or at least conformationally accessible, for interaction with nucleic acids of a unique, sequence-conferred deformation. Protein-bound Lewis acids may contact the phosphate backbone of nucleic acids directly by hydrogen bonding or indirectly via a bridging water molecule. Alternatively, a metal ion such as calcium may bridge a carboxylate of the protein and a phosphate of the nucleic acid, as

(1) (a) Matthews, B. W. *Nature* **1988**, *335*, 294–295. (b) Otwinowski, Z.; Schevitz, R. W.; Zhang, R.-G.; Lawson, C. L.; Joachimiak, A.; Marmorstein, R. Q.; Luisi, B. F.; Sigler, P. B. *Nature* **1988**, *335*, 321–329. (c) Anderson, J. E.; Ptashne, M.; Harrison, S. C. *Nature* **1987**, *326*, 846–852.

(2) Gandour, R. D. *Bioorg. Chem.* **1981**, *10*, 169–176.

(3) Carrell, C. J.; Carrell, H. L.; Erlebacher, J.; Glusker, J. P. *J. Am. Chem. Soc.* **1988**, *110*, 8651–8656.

(4) (a) Wiberg, K. B.; Laidig, K. E. *J. Am. Chem. Soc.* **1987**, *109*, 5935–5943. (b) Peterson, M. R.; Csizmadia, I. G. *J. Am. Chem. Soc.* **1979**, *101*, 1076–1079.

(5) (a) Allen, F. H.; Kennard, O.; Taylor, R. *Acc. Chem. Res.* **1983**, *16*, 146–153. (b) Allen, F. H.; Bellard, S.; Brice, M. D.; Cartwright, B. A.; Doubleday, A.; Higgs, H.; Hummelink, T.; Hummelink-Peters, B. G.; Kennard, O.; Motherwell, W. D. S.; Rodgers, J. R.; Watson, D. G. *Acta Crystallogr.* **1979**, *B35*, 2331–2339.

(6) For example, see: (a) Vedani, A.; Dunitz, J. D. *J. Am. Chem. Soc.* **1985**, *107*, 7653–7658. (b) Murray-Rust, P.; Stallings, W. C.; Monti, C. T.; Preston, R. K.; Glusker, J. P. *J. Am. Chem. Soc.* **1983**, *105*, 3206–3214. (c) Murray-Rust, P.; Glusker, J. P. *J. Am. Chem. Soc.* **1984**, *106*, 1018–1025. (d) Ramasubbu, N.; Parthasarathy, R.; Murray-Rust, P. *J. Am. Chem. Soc.* **1986**, *108*, 4308–4314. (e) Burley, S. K.; Petsko, G. A. *Science* **1985**, *229*, 23–28.

(7) (a) Jack, A.; Ladner, J. E.; Rhodes, D.; Brown, R. S.; Klug, A. *J. Mol. Biol.* **1977**, *111*, 315–328. (b) Holbrook, S. R.; Sussman, J. L.; Warrant, R. W.; Church, G. M.; Kim, S.-H. *Nucleic Acids Res.* **1977**, *4*, 2811–2820. (c) Fresco, J. R.; Adams, A.; Ascione, R.; Henley, D.; Lindahl, T. *Cold Spring Harbor Symp. Quant. Biol.* **1966**, *31*, 527–537.

(8) Latham, J. A.; Cech, T. R. *Science* **1989**, *245*, 276–282.

(9) (a) Jordan, S. R.; Pabo, C. O. *Science* **1988**, *242*, 893–899. (b) Wolberger, C.; Dong, Y.; Ptashne, M.; Harrison, S. C. *Nature* **1988**, *335*, 789–795. (c) McClarin, J. A.; Frederick, C. A.; Wang, B.-C.; Greene, P.; Boyer, H. W.; Grable, J.; Rosenberg, J. M. *Science*, **1986**, *234*, 1526–1541. (d) Aggarwal, A. K.; Rodgers, D. W.; Drottar, M.; Ptashne, M.; Harrison, S. C. *Science* **1988**, *242*, 899–907.

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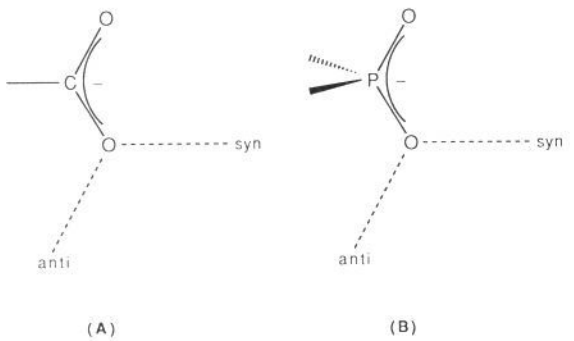

Figure 1. (A) Stereochemistry of metal ion or hydrogen bond donor geometry with the carboxylate described as syn or anti as the Lewis acid interacts with one of the lone electron pairs on oxygen. These stereochemical designations also describe the interactions of the phosphinyl monoanion with Lewis acids (B).

recently hypothesized by Sigler and colleagues.[1b] Herein, we detail the preferred stereochemistry of Lewis acid interactions with the phosphate monoanion.

Methods

The CSD was installed on a VAXstation 3500, and all database manipulations utilized standard CSD software. Our first search of the database employed a connectivity algorithm with the program CONNSER. This connectivity search targeted a phosphorus added to at least two oxygens; additionally, only those structures were retrieved in which a metal ion or hydrogen bond donor (H–O or H–N) was also present in the crystallographic unit cell. The program GEOSTAT89 was used to eliminate those structures in which the phosphinyl oxygen–metal ion distance was greater than 2.50 Å, as well as those structures in which phosphinyl–hydrogen bond donor (i.e., phosphinyl oxygen–hydrogen) distances were greater than 2.10 Å. Additionally, structures with crystallographic R factors greater than 0.15 were excluded. We found that 88% of the retrieved phosphinyl–metal ion structures displayed $R \leq 0.10$ and 79% of the retrieved structures displayed $R \leq 0.08$. Likewise, we found that 98% of the retrieved phosphinyl–hydrogen bond donor structures displayed $R \leq 0.10$ and 96% of the retrieved structures displayed $R \leq 0.08$. The inclusion of those structures with $0.08 \leq R \leq 0.15$ in the coordinate retrievals did not alter stereochemical statistics.

Coordinate entries that survived the connectivity and geometry screening processes were examined individually on a Tektronics 4207 terminal with the program PLUTO89. We eliminated each structure where possible stereochemical bias might have been imposed upon the complex. Specifically, chelate interactions involving the phosphinyl group and another Lewis base on the same molecule (sometimes another phosphinyl group) were eliminated, since a dual interaction might skew the preferred stereochemistry at a single phosphinyl group. Additionally, chelates involving dimetallic clusters were eliminated (e.g., a single phosphinyl interacting with a Pt–Pt or Sb–H_2O–Sb couple), since again such a feature might skew the phosphinyl coordination stereochemistry. Coordinate entries were screened in order to excise interactions involving dianionic phosphates, i.e., R–PO_3^{2-}, even though these interactions paralleled those involving the phosphinyl monoanion. Our goal was to determine the preferred geometry of a single phosphate as it interacts with a single Lewis acid.

A bibliographic search of the CSD using the program BIBSER, independent of that performed with the chemical connectivity program CONNSER, was conducted in order to verify that all relevant coordinate entries had been retrieved from the CSD. We searched for those entries that contained the fragment "phos" in their formal names, and we ultimately produced the same set of coordinate retrievals as output from CONNSER.

Scatterplots were made by superimposing the tetrahedral phosphates of each retrieval and allowing the coordinated metal ions or hydrogen bond donors to "ride" into proper position relative to the reference phosphate. This procedure was performed on an Evans and Sutherland PS300 interfaced with a VAX 11/750 using the software package FRODO.[10] Subsequently, two symmetry operations were applied to complexed metal ions or hydrogen bonds according to the mirror symmetry of the phosphinyl group: the first operation above and below the plane of the phosphinyl anion and the second operation across the plane bisecting the anion. Coordinate sets containing symmetric equivalent metal ion or hydrogen atom positions were used to generate the final scatterplots. Contour plots, which allow for qualitative visualization of most probable regions of coordination space, were generated with software CELL10, GENAT, GENMAP, and FIXMAP[11] kindly provided by Dr. H. L. Carrell, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia.

Table I. Phosphinyl–Metal Ion Interactions

metal	entries	syn	% syn	anti	% anti
Na	16	11	69	5	31
Cu	13	7	54	6	46
Ca	12	9	75	3	25
Zn	12	8	67	4	33
Sn	11	5	45	6	55
Cr	8	8	100	0	0
Mg	5	2	40	3	60
Re	5	5	100	0	0
U	5	3	60	2	40
Dy	4	1	25	3	75
Mn	4	2	50	2	50
Sr	4	2	50	2	50
Mo	3	2	67	1	33
Ni	2	0	0	2	100
Sb	2	2	100	0	0
Co	1	0	0	1	100
Ir	1	1	100	0	0
total	108	68	63	40	37

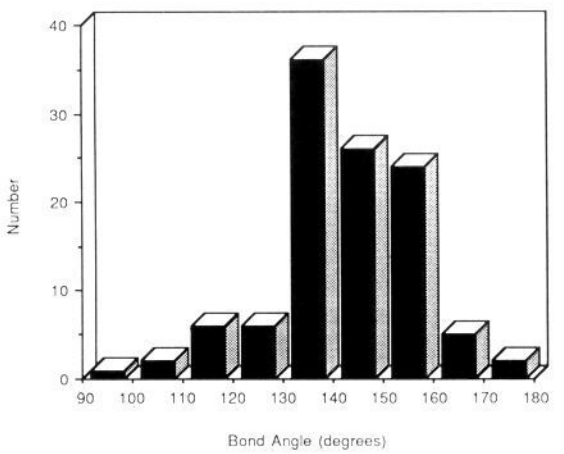

Figure 2. Histogram of phosphinyl–metal ion bond angles. The average P=O—M^{n+} angle is 141 ± 14°.

Results and Discussion

Metal Ions. There are 108 unique and independent phosphinyl–metal ion interactions in the CSD, extracted from 48 coordinate entries, that satisfy the structural criteria outlined above. The CSD reference code and numerical data for each retrieval are included in the supplementary material. The identity, number, and stereochemical statistics for these interactions are reported in Table I. Retrievals consist of phosphinic acids (43%), phosphates (34%), and a handful of other phosphinyl derivatives (≤12% each). Each of these phosphinyl derivatives displays consistently similar stereochemistry as it interacts with metal ions. Our discussion, based on the stereochemical statistics of all phosphinyl–Lewis acid interactions, is grounded in the pertinence of these statistics to biologically important phosphate diesters.

We observe no symmetrically bidentate phosphinyl–metal ion interactions; nevertheless, as noted for carboxylate–metal ion interactions,[3] syn coordination stereochemistry is preferred over anti. In view of this result, it is interesting that a recent report by Hendry and Sargeson, based on work with small model systems, concludes that four-membered phosphate–metal ion chelates are unfavorable and irrelevant in biological systems.[12] For all metals,

(10) Jones, T. A. In *Computational Crystallography*; Sayre, D., Ed.; Clarendon: Oxford, 1982; pp 303–317.

(11) Rosenfield, R. E., Jr.; Swanson, S. M.; Meyer, E. F., Jr.; Carrell, H. L.; Murray-Rust, P. *J. Mol. Graphics* **1984**, *2*, 43–46.

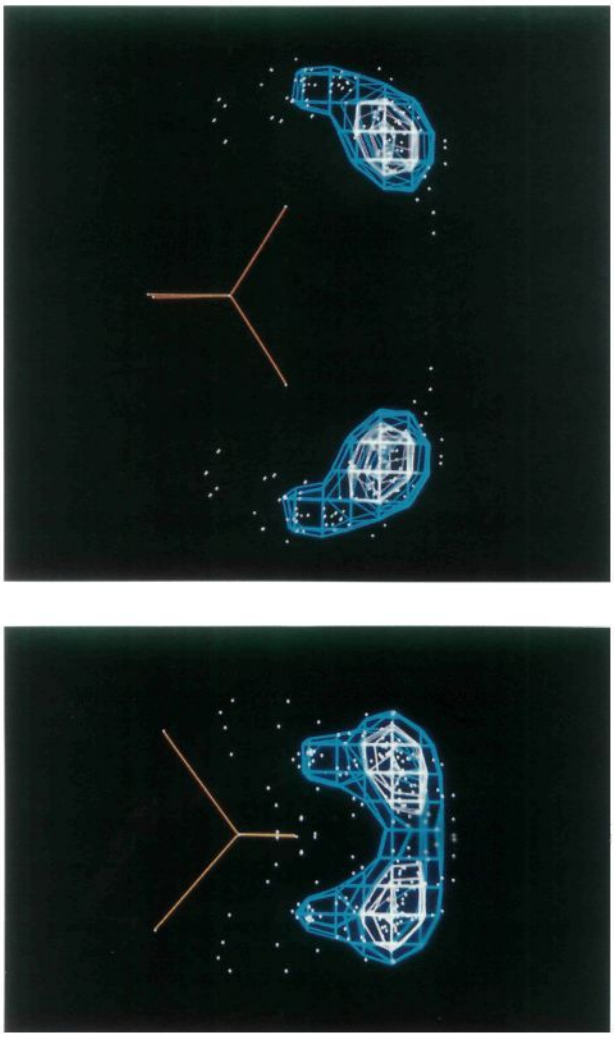

Figure 3. Contour map, generated at two arbitrary density levels, superimposed on a scatterplot of all phosphinyl–metal ion interactions. Two perpendicular orientations (top) and (bottom) are shown. The higher contour density (magenta) indicates where most metal ions tend to cluster as they interact with the phosphinyl group; symmetry-equivalent positions for each interaction are shown above and across the phosphinyl plane. Syn stereochemistry is preferred over anti by 63% to 37% for these 108 unique and independent interactions, which show a preference for out-of-plane geometry. Note the complete absence of symmetrically bidentate metal coordination by PO_2^-.

our survey reveals that syn unidentate coordination by the phosphinyl group is preferred over anti by 63% to 37%. The average P═O—M^{n+} angle for all retrievals is 141 ± 14°; a histogram of this angular distribution is found in Figure 2. A countour plot superimposed on a scatterplot of these interactions is presented in Figure 3. While our statistics do not allow us to elaborate on the differences in coordination preference among the different metal ions due to a small number of individual examples, we note that all metal ions tend to cluster within the same regions of coordination space regardless of their identities. The bell-shaped histogram of Figure 2 and the corresponding density of interactions observed in Figure 3 reflect this clustering effect. Interestingly, phosphate–metal ion interactions observed in tRNA structures are generally in accord with these statistics.[7]

An intriguing contrast between carboxylates and phosphates, as they interact with metal ions, can be made. In carboxylate–metal ion complexes, the metal ion typically prefers to be in the plane of the carboxylate group.[3] However, the metal ion engaged

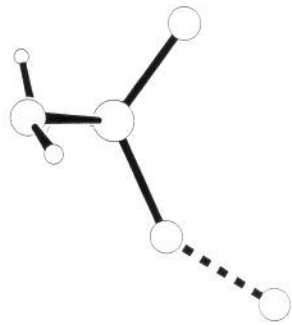

Figure 4. Fully optimized $H_2PO_4^-$–Li^+ interaction as calculated by ab initio methods at the 3-21G* level. Small circles denote hydrogens. A dashed line represents the PO_2^-–Li^+ interaction, in which the P═O—Li^+ angle is 146°.

by phosphate prefers a location that is 0.9 ± 0.5 Å out of the phosphinyl plane (see Figure 3, bottom). Clearly, then, the phosphate monoanion cannot be regarded as an electronic analogue of a carboxylate.

Molecular orbital theory may provide an explanation for these electronic differences. Kutzelnigg and colleagues have shown, in detailed ab initio calculations, that the semipolar P═O double bond of R_3P═O is electronically different from the C═O double bond, e.g., as found in H_2C═O.[13] These investigators find that the P═O double bond is more properly designated a partial triple bond, i.e., as one full σ bond and two mutually perpendicular, half π bonds (formed by back-bonding between the electrons of oxygen and the empty d orbitals of phosphorus). Theoretically, then, there should be a lone electron pair nearly opposite the P═O bond on oxygen. We propose that these molecular orbital considerations extend to the phosphinyl monoanion, with the conclusion that the actual "hybridization" of phosphinyl oxygen atoms is somewhere between sp and sp^2 due to the dative character of P═O and thus O═P—O^- systems. This molecular orbital explanation is in accord with the absence of symmetrically bidentate phosphinyl–metal ion interaction in our analysis.

Molecular orbital calculations performed in our laboratory with GAUSSIAN 82[14] and the 3-21G* basis set[15] are consistent with the statistical results drawn from the CSD. The geometry of the model system comprised of $H_2PO_4^-$ and Li^+ was fully optimized. The optimized structure shows that Li^+ binds with syn orientation to the P═O bond at an angle of 146° (Figure 4). Results obtained with the model system $H_2PO_4^-$ and Be^{2+} indicate that the minimum-energy geometry is more nearly in line with the P═O bond. Attempts to minimize the energy of $H_2PO_4^-$ coordinated in bidentate fashion to Li^+ were unsuccessful in finding a completely optimized structure. Constraining the phosphate to bidentate interactions with each metal ion resulted in significantly higher energies than those obtained for optimized, unidentate structures. These results imply that the metal ion interacts with a nearly pure sp lone electron pair on oxygen. However, our stereochemical statistics lead us to believe that the phosphinyl oxygens are between sp- and sp^2-hybridized—recall that the average P═O—M^{n+} angle is 141°. Further calculations are required, however, before a "hybridization" effect can be properly quantified. Moreover, since metal ions are observed to cluster in out-of-plane regions, stereoelectronic effects (perhaps directed by the other two substituents on the phosphinyl phosphorus atom) may affect the orientation of the phosphinyl oxygen lone electron pair. Therefore, we reinforce our conclusion that the preferred interaction of the PO_2^- group with a single metal ion is not bidentate; instead, the preferred coordination geometry tends toward unidentate (Figure 5).

(12) Hendry, P.; Sargeson, A. M. *J. Am. Chem. Soc.* **1989**, *111*, 2521–2527.

(13) (a) Kutzelnigg, W. *Pure Appl. Chem.* **1977**, *49*, 981–1000. (b) Wallmeier, H.; Kutzelnigg, W. *J. Am. Chem. Soc.* **1979**, *101*, 2804–2814.

(14) (a) Binkley, J. S.; Frisch, M.; Krishnan, R.; DeFrees, D. J.; Schlegel, H. B.; Whiteside, R.; Fluder, E.; Seeger, R.; Pople, J. A. GAUSSIAN82, Release H; Carnegie-Mellon University: Pittsburgh, PA, 1982. (b) Binkley, J. S.; Pople, J. A.; Hehre, W. J. *J. Am. Chem. Soc.* **1980**, *102*, 939–947.

(15) Pietro, W. J.; Francl, M. M.; Hehre, W. J.; DeFrees, D. J.; Pople, J. A.; Binkley, J. S. *J. Am. Chem. Soc.* **1982**, *104*, 5039–5048.

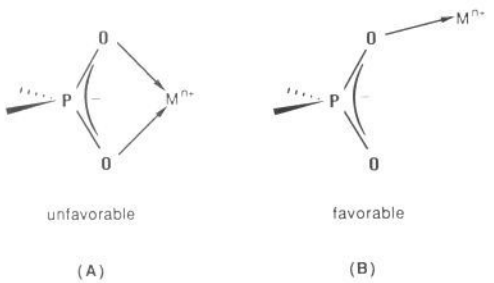

Figure 5. Stereochemical analysis suggests that symmetrically bidentate phosphinyl–metal ion interaction is unfavorable (A) relative to a unidentate, syn geometry which tends toward in-line and out-of-plane orientation with regard to the phosphinyl moiety (B).

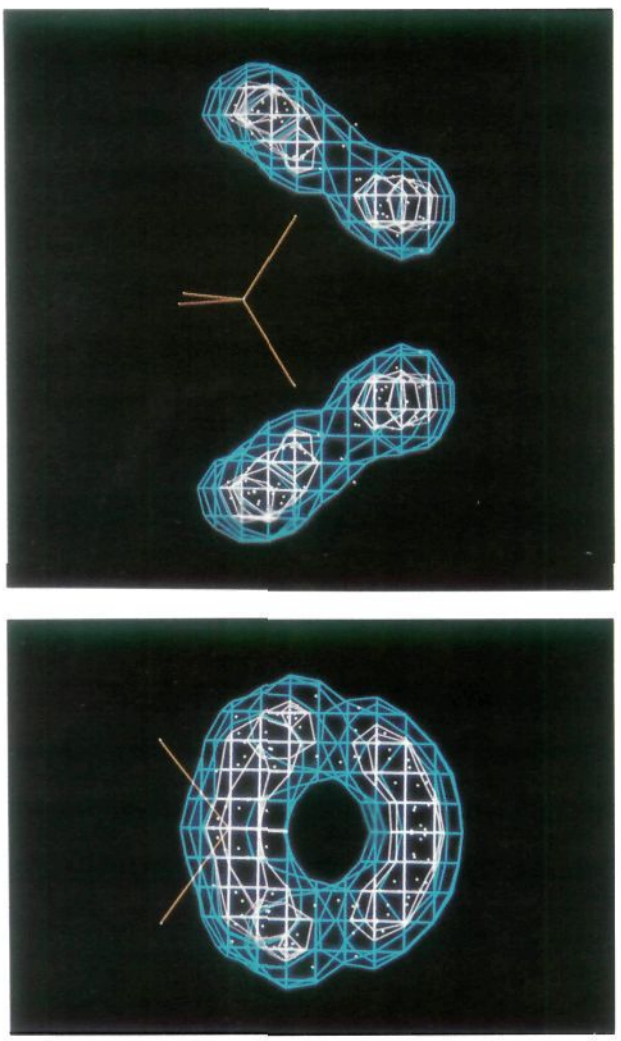

Figure 6. Contour map, generated at two arbitrary contour levels, superimposed on a scatterplot of all 56 phosphinyl–hydrogen bond donor interactions. Symmetry-equivalent positions for each interaction are shown above and across the phosphinyl plane; two perpendicular orientations (top) and (bottom) are shown. Anti stereochemistry is slightly preferred over syn by 54% to 46% for these unique and independent interactions, which show no preference for in-plane geometry. In contrast with phosphinyl–metal ion interactions, there are no direct, in-line interactions with the P=O bond as revealed by the annulus in the hydrogen distribution (bottom).

Hydrogen Bond Donors. *There are 56 unique and independent* phosphinyl–hydrogen bond interactions retrieved from 44 coordinate entries of the CSD that satisfy our structural criteria. The CSD reference code and numerical data for each retrieval are included in the supplementary material. These interactions are

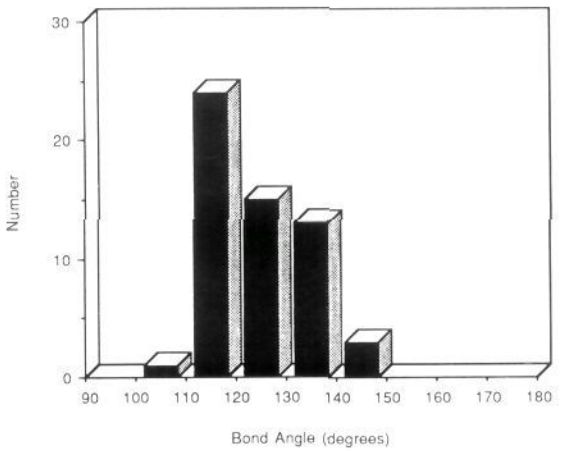

Figure 7. Histogram of phosphinyl–hydrogen bond donor angles. The average P=O—H angle is 124 ± 9°.

drawn from entries consisting of phosphates (70%), phosphonates (27%), and phosphinic acids (3%). Each of these phosphinyl derivatives displays consistently similar stereochemistry as it interacts with hydrogen bond donors; a scatterplot of phosphinyl–hydrogen bond donor interactions is presented in Figure 6. It is interesting to note the annulus of hydrogens about the P=O axis in Figure 6 which is absent from phosphinyl–metal ion interactions (Figure 3). The average P=O—H angle is 124 ± 9°, and a histogram of this angular distribution is found in Figure 7. Hydrogen bond donors do not display a preference for in-plane interactions with the phosphinyl group, nor do they display an apparent preference for syn versus anti orientation to the phosphinyl group. Actually, there is a slight excess of anti-oriented interactions (54%) relative to syn-oriented interactions (46%). Perhaps this different ratio reflects the weaker charge–dipole interaction of phosphate with a hydrogen bond donor relative to the stronger charge–charge interaction of phosphate with a metal ion. Baker and Hubbard note that hydrogen bonds to C=O in proteins do not strongly prefer in-plane geometry,[16] so perhaps this too implies some degree of isotropy for charge–dipole and dipole–dipole interactions. Murray-Rust and Glusker find predominantly in-plane character, accompanied by significant out-of-plane scatter, for hydrogen bonds to the carbonyl in a survey of the CSD.[6c]

Given that our results for phosphate–hydrogen bond donor stereochemistry are relevant to the evaluation of protein–nucleic acid interactions,[1b,c,9] we acquired the coordinates of phage 434,[9d] λ,[9a] and *trp*[1b] repressor–operator complexes in order to analyze the stereochemical distribution of hydrogen bonds between DNA phosphates and protein hydrogen bond donors. A total of 58 independent interactions were extracted from these coordinate sets. The scatterplot of Figure 8 reports the distribution of presumed hydrogen bonds (inferred from *non-hydrogen* donor atom positions) as extracted from these structures. The average P=O—donor angle is 124 ± 18°. In accord with our results from CSD analysis, we make the following observations: (1) the presumed hydrogen bond distribution derived from the repressor–operator complexes does not display a large preference for syn (53%) versus anti (47%) stereochemistry; (2) the distribution of presumed hydrogen bonds in the repressor–operator complexes shows no particular preference for in-plane interaction with the phosphinyl portion of DNA phosphates; and (3) linear P=O—donor angles are absent in both the protein–DNA complexes and the CSD retrievals. We emphasize, however, that the conclusions drawn from the structures of protein–nucleic acid models must be regarded as tentative due to the resolution of the electron density maps into which atomic models have been built (1.9–2.5 Å). Resolution extension and refinement of each repressor–operator

(16) Baker, E. N.; Hubbard, R. E. *Prog. Biophys. Mol. Biol.* **1984**, *44*, 97–179.

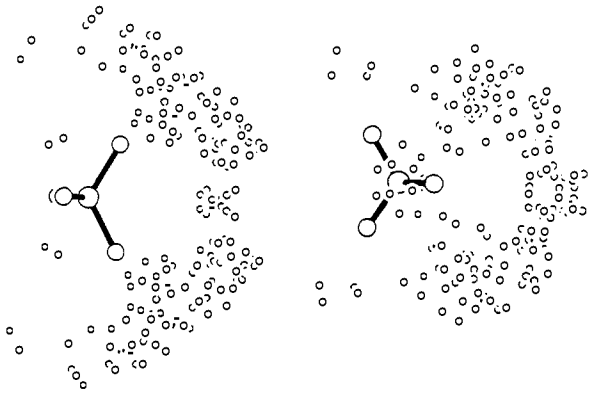

Figure 8. Two perpendicular orientations (left and right) of presumed phosphate–hydrogen bond donor interactions showing symmetry equivalents above and across the phosphinyl plane. These 58 unique interactions are extracted from the structures of phage 434, λ, and *trp* repressor–operator complexes. Each atom in the distribution represents the *non-hydrogen* donor atom; hydrogen positions can only be inferred from macromolecular X-ray crystallographic studies. No great preference for syn versus anti stereochemistry is observed (of 58 independent interactions, 53% display syn and 47% display anti stereochemistry), and there is apparently no preference for in-plane interaction. It is intriguing that this scatterplot, derived from macromolecular crystallographic results, shows a lack of linear P=O donor interactions; this phenomenon is common to the distribution derived from CSD analysis (Figure 6).

model may strengthen these tentative observations.

Phosphinyl Derivatives as Transition-State Analogues. Tetrahedral phosphinic acids,[17] phosphonamidates,[18] and phosphonates[18] have been utilized as analogues of the proteolytic or esterolytic transition state/intermediate for enzymes of the zinc and aspartic protease families. From the correlation between inhibitor K_i values and the related substrate K_m/k_{cat} values, the transition-state analogy has been elegantly demonstrated.[18b] Our stereochemical analysis provides an interesting perspective upon the mode of action of these compounds. For instance, our statistical results suggest, and the molecular orbital results of Kutzelnigg[13] imply, that symmetrically bidentate metal ion coordination is unfavorable for the tetrahedral phosphonate or phosphonamidate of the zinc protease inhibitor.[18] Hence, we propose that there are electronic limitations upon the analogy between the phosphinyl monoanion and the monoanionic, promoted-water hydrolytic transition state. We have shown that the phosphinyl moiety tends toward unidentate coordination to a metal ion—this conclusion is in accord with the structures of binary enzyme–inhibitor complexes involving thermolysin[19] and carboxypeptidase A,[20] where phosphinyl-containing inhibitors generally tend toward asymmetric, unidentate interactions. However, the *bidentate* interaction of the tetrahedral intermediate (and its flanking transition states) with zinc is proposed for the proteolytic mechanisms of these zinc proteases.[21] How well can the intermediate sp–sp^2 hybridized oxygens of the phosphinyl monoanion mimic the intermediate sp^3–sp^2 hybridized oxygens of a proteolytic transition state? This question is relevant to the generation of catalytic antibodies (esterases, proteases) by eliciting them against phosphinyl-derived haptens.[22] Although the X-ray crystallographic structure determination in Wilson's group[23] of the abzyme–transition-state analogue complex Fab–P3 may be interpretable in light of the phosphinyl–hydrogen bond geometric preferences herein outlined, we suggest that molecular orbital differences between the phosphinyl group and the hydrolytic transition state it approximates may ultimately pose an upper limit to the use of phosphinyl derivatives as efficient abzyme haptens. Currently, abzymes display only modest rate accelerations (ca. 10^3) of hydrolytic reactions as compared with uncatalyzed reaction rates.[22]

Alternatively, given that the transition-state analogy holds for the phosphinyl-containing zinc protease inhibitors,[18] it may be possible that the actual transition state of the hydrolytic reaction does not tend toward symmetrically bidentate coordination to the active-site zinc ion.

Summary and Conclusions

The monoanionic phosphinyl portion of the phosphate group prefers syn, unidentate coordination to metal ions; this coordination shows some preference for out-of-plane geometry with respect to the phosphinyl group. Phosphinyl–hydrogen bond donor interactions display a slight preference for anti interaction with regard to the phosphinyl moiety. Additionally, hydrogen bond donors do not show a preference for in-plane interaction with the phosphinyl group. Interestingly, these stereochemical results may be contrasted with those reported by other research groups for carboxylate–Lewis acid interactions: carboxylates tend toward syn, in-plane interactions.[2-4] These fundamental differences arise from the dative character of the P=O bond, which is considered as one full σ bond and two half π bonds.[13] This arrangement theoretically gives rise to sp or intermediate sp–sp^2 hybridization at the phosphinyl oxygen, which requires that a lone electron pair be directed at an angle between 120° and 180° from the P=O bond; it is this lone pair which complexes Lewis acids.

In conclusion, given that phosphate–Lewis acid interactions govern nucleic acid structure to some degree, we have demonstrated that there is a preferred stereochemistry for individual phosphinyl–Lewis acid interactions that, in the aggregate of a nucleic acid structure or a protein–nucleic acid complex, may ultimately confer specificity to functional and recognition events.

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Supplementary Material Available: Two tables giving CSD reference codes and structural parameters for coordinate retrievals involving phosphinyl–metal ion and phosphinyl–hydrogen bond donor interactions (4 pages). Ordering information is given on any current masthead page.

(17) Bartlett, P. A.; Kezer, W. B. *J. Am. Chem. Soc.* **1984**, *106*, 4282–4283.
(18) (a) Jacobsen, N. E.; Bartlett, P. A. *J. Am. Chem. Soc.* **1981**, *103*, 654–657. (b) Bartlett, P. A.; Marlowe, C. K. *Biochemistry* **1983**, *22*, 4618–4624. (c) Bartlett, P. A.; Marlowe, C. K. *Science* **1987**, *235*, 569–571. (d) Bartlett, P. A.; Marlowe, C. K. *Biochemistry* **1987**, *26*, 8553–8561.
(19) (a) Tronrud, D. E.; Holden, H. M.; Matthews, B. W. *Science* **1987**, *235*, 571–574. (b) Holden, H. M.; Tronrud, D. E.; Monzingo, A. F.; Weaver, L. H.; Matthews, B. W. *Biochemistry* **1987**, *26*, 8542–8553.
(20) (a) Christianson, D. W.; Lipscomb, W. N. *J. Am. Chem. Soc.* **1986**, *108*, 545–546. (b) Christianson, D. W.; Lipscomb, W. N. *J. Am. Chem. Soc.* **1988**, *110*, 5560–5565.
(21) (a) Matthews, B. W. *Acc. Chem. Res.* **1988**, *21*, 333–340. (b) Christianson, D. W.; Lipscomb, W. N. *Acc. Chem. Res.* **1989**, *22*, 62–69.
(22) (a) Tramontano, A.; Janda, K. D.; Lerner, R. A. *Science* **1986**, *234*, 1566–1570. (b) Pollack, S. J.; Jacobs, J. W.; Schultz, P. G. *Science* **1986**, *234*, 1570–1573.
(23) Stura, E. A.; Fieser, G. G.; Rini, J. M.; Wilson, I. A. Scientific Report; Research Institute of Scripps Clinic: La Jolla, CA, 1987; Vol. 13, p 178.