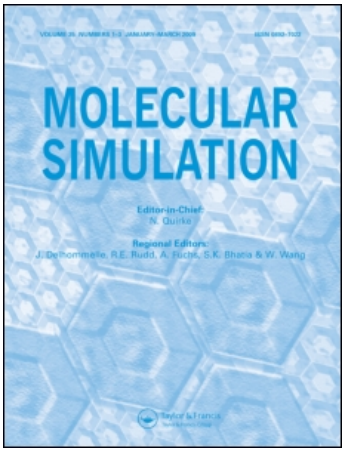

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The structure of N δ -(N '-sulfodiaminophosphinyl)- l -ornithine and its binding to ornithine transcarbamoylase: A quantum chemical study

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THE STRUCTURE OF N^{δ}-(N'-SULFODIAMINOPHOSPHINYL)-L-ORNITHINE AND ITS BINDING TO ORNITHINE TRANSCARBAMOYLASE: A QUANTUM CHEMICAL STUDY

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The structure of N^{δ}-(N'-Sulfodiaminophosphinyl)-L-ornithine (PSOrn) in complex with the enzyme ornithine transcarbamoylase (OTCase) was recently characterised by Langley *et al.* [D.B. Langley, M.D. Templeton, B.A. Fields, R.E. Mitchell and C.A. Collyer, J. Biol. Chem., 275 (2000) 20012] using X-ray diffraction techniques. In this work, the interaction of PSOrn with the arginine residues of OTCase is modelled using density functional theory, with an emphasis on characterising the mechanism of binding between PSOrn, an inhibitor, and the enzyme. For the purposes of this study, the interaction of PSO, an analogue of PSOrn (obtained by replacing a $(CH_2)_3CH(CO_2^-)(NH_3^+)$ side chain by methyl) with one and two arginine (Arg) molecules are investigated. The $PSO\cdots(Arg)_2$ trimer is found to be strongly bound, by ~ 171 kJ mol^{-1}, due to the presence of four hydrogen bonds in addition to a large ionic interaction between a dinegative PSO^{2-} and protonated arginines. The computed geometry is consistent with the X-ray structure and the large binding energy is consistent with the observation that PSOrn is a powerful inhibitor. Furthermore, in agreement with the proposals of Langley *et al.*, the most stable bound form of PSO is found to be an imino type tautomer. The population analyses that were carried out on PSO suggest that PN, PO, SN and SO bonds, as in a range of other systems, are generally either single or semipolar bonds.

Keywords: N^{δ}-(N'-Sulfodiaminophosphinyl)-L-ornithine; PSOrn; Phaseolotoxin; Enzyme/inhibitor interactions; Hydrogen bonding; B3LYP DFT computations

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INTRODUCTION

N^{δ}-(*N'*-Sulfodiaminophosphinyl)-L-ornithine (PSOrn) is the active component of phaseolotoxin, which in turn is derived from a toxin produced by *Pseudomonas syringae* pv. *phaseolicola*. PSOrn has been found to bind to the *E.coli* enzyme ornithine transcarbamoylase (OTCase) with a dissociation constant of 1.6×10^{-12} M at 37°C, pH = 8 [1]. OTCase catalyses the reaction of carbamoyl phosphate with L-ornithine, forming L-citrulline and phosphate and forms part of the urea cycle for mammals. It is also involved in the synthesis of arginine by plants and bacteria. The binding of PSOrn to OTCase irreversibly halts this catalysis, resulting in cell death.

The X-ray crystal structure of PSOrn within the enzyme has been determined at 1.8 Å resolution by Langley *et al.* [1]. The resulting heavy atom backbone of this molecule is shown in Fig. 1. Since crystal structures refined at this resolution define only the positions of non-hydrogen atoms, the chirality, tautomeric form and the ionization state of the bound inhibitor could at best be inferred from the structural data using chemical considerations. This study aims to investigate, using the methods of computational quantum chemistry, the chemical identity of PSOrn in both free and bound states, determine their relative stabilities and clarify the nature of bonding both within the inhibitor and between the enzyme and inhibitor.

Langley *et al.* [1] proposed that PSOrn acts as a transition state analogue within the enzyme, i.e. it adopts the same conformation and forms the same type of hydrogen bonds with the enzyme as in the suggested carbamoyl phosphate + L−ornithine transition state. They examined the self-consistency of possible hydrogen bonding networks at the enzyme active site and concluded that the most

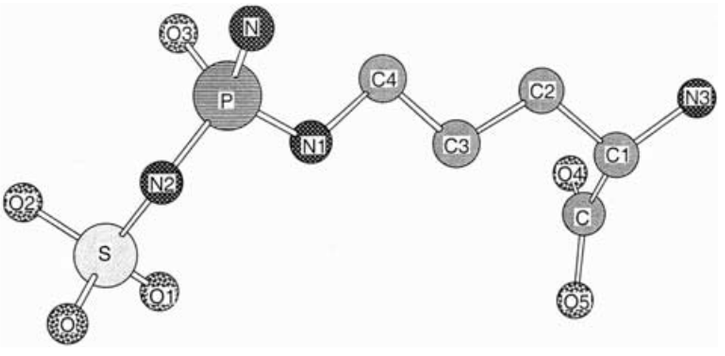

FIGURE 1 Structure of PSOrn within OTC as determined by X-ray diffraction [1].

likely chemical form of the bound inhibitor is a doubly ionized "imino" tautomer and that the phosphotriamide is the *R* enantiomer as shown in Fig. 2, along with the substrates, proposed transition states and a more conventional amino form of the inhibitor. The observed potent inhibitory activity of PSOrn could thus be rationalized because this species is a structural mimic of the substrates in a proposed transition state.

Conventional chemical wisdom suggests that free PSOrn would be more stable in the amino form, i.e. with the P–N–S nitrogen protonated. Moreover, such protonation would not necessarily preclude hydrogen bond donation to nitrogen, given the presence of a lone pair on N. The current investigations were undertaken with the primary aim of elucidating the nature of the interaction between PSOrn and some of the important enzyme residues, in particular the hydrogen bonding of an arginine to the P–N–S nitrogen of the inhibitor. To reduce computational costs the $(CH_2)_3CH(NH_3^+)$, (CO_2^-) side-chain of PSOrn has been replaced by a methyl group. The relative stabilities of several tautomeric amino and imino forms of the resulting neutral model compound are investigated by density functional theory, followed by similar studies on adducts of these with one and two arginine molecules. In addition to providing information on the relative stabilities of the amino and imino forms of the inhibitor in both free and bound forms, these studies also yield charge distribution data and thus some insight into the nature of bonding within the inhibitor as well as between inhibitor and enzyme.

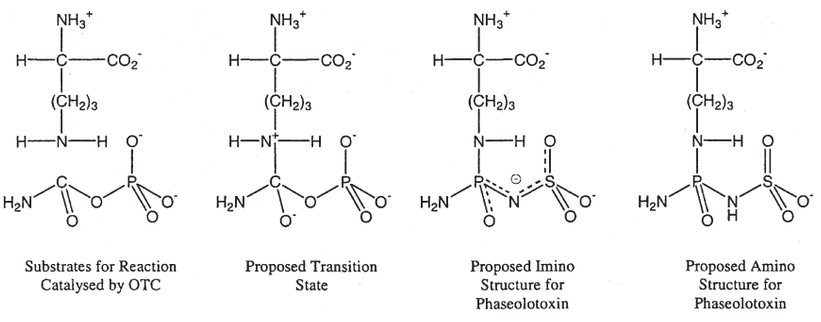

FIGURE 2 Imino and amino tautomers of PSOrn as transition state analogues for the OTC catalysed reaction, as proposed by Langley *et al.* [1].

METHODS

In the work reported in this paper, neutral PSOrn is represented by model compound, denoted PSO, obtained by replacing the $(CH_2)_3CH(NH_3^+)(CO_2^-)$ side-chain of PSOrn by a methyl group. The structure of an amino form of PSO is shown schematically in Fig. 3. As indicated by the X-ray data, the side-chain is not directly involved in the binding of PSOrn to the arginine residues. Therefore, the above simplification of the inhibitor is not expected to significantly affect the inhibitor/arginine interactions, especially since the $CH(NH_3^+)(CO_2^-)$ group, being at the end of a fully extended saturated alkyl chain, would only marginally affect the covalent bonding pattern (and hence electron density) within the "active" PSO moiety (either via through-space or through-bond interactions).

The quantum chemical calculations on PSO and the PSO/arginine adducts were carried out using density functional theory, utilizing the B3LYP exchange-correlation hybrid functional [2] and the 6-31G(d) basis set. Full geometry optimisations were performed as well as constrained optimisations, where only the hydrogen coordinates were allowed to relax, while the heavy atom coordinates were constrained at the X-ray values. For a number of species, the energies were recalculated using the fully polarised 6-31G(d,p) basis. The inclusion of polarisation functions on the hydrogens resulted in effectively negligible changes in the relative energies. Due to computer resource limitations vibrational frequencies and hence zero point energy (ZPE) corrections were not, in general, computed. On the basis of ZPE computations on two zwitterionic dimers: Zw1 and Zw2 (see Fig. 7), and their constituent PSO and arginine monomers, the dissociation energy of a dimer would be reduced by $\sim 5-10\,\mathrm{kJ\,mol^{-1}}$, viz. up to $\sim 15\%$, by the inclusion of ZPE. As this work does not aim to produce energies of chemical accuracy, the omission of ZPE is justifiable.

All calculations were carried out using the GAUSSIAN98 programs [3] on DEC alpha 600/5/333 and COMPAQ XP1000/500 workstations of the Theoretical Chemistry group at the University of Sydney and the 64 processor SGI Origin

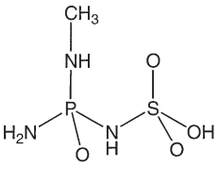

FIGURE 3 The structure (connectivity) of PSO, the model compound for PSOrn.

2400 of the Australian Centre for Advanced Computing and Communications (ac3).

RESULTS AND DISCUSSION

Free (model) Inhibitor

All chemically reasonable tautomers of the model inhibitor PSO were considered in an effort to locate the most stable tautomer and to quantify their relative stabilities. Free PSO, and thus PSOrn, has the potential to form intramolecular hydrogen bonds, which in all probability will have a considerable effect on these stabilities. The optimised structures of five amino and three imino tautomers of neutral PSO are shown in Figs. 4 and 5, along with their relative energies. Although it may not be immediately obvious from these figures, the geometries of the heavy atom backbones of the various tautomers are quite different, especially in the angles. The variation is attributed, in part at least, to the effects of intramolecular hydrogen bonding, which of course, are tautomer dependent. Constraining the heavy atom coordinates to their X-ray values effectively eliminates most of the intramolecular hydrogen bonds at an energy cost of $\sim 400\,\mathrm{kJ\,mol^{-1}}$. As only part of this energy could be reasonably attributed to the hydrogen bonds, the relaxation energy associated with optimisation of the (covalent) bond distances and bond angles is obviously substantial. In light of such demonstrated sensitivity of the energy to relatively small variations in the geometry, there is clearly a need for fully relaxed calculations, i.e. we cannot rely entirely on the results of constrained computations.

According to the computed equilibrium energies shown in Figs. 4 and 5, the imino tautomers are substantially less stable than the amino forms. This was expected, as in the former the S–N–P nitrogen would have two lone pairs of electrons and a formal negative charge, so to stabilise such a structure considerable charge delocalisation would be needed. As will be shown later, the sulfur and phosphorus atoms in their respective environments in PSO cannot participate in π bonding to any appreciable degree, i.e. no significant π charge delocalisation occurs. This is contrary to the implications of the Lewis structure of the imino form of PSOrn in Fig. 2. Hence the marked difference in stabilities between amino and imino tautomers.

Bound (model) Inhibitor

The crystal structure of PSOrn in OTCase indicates that the inhibitor is hydrogen bonded to an arginine residue (Arg57), as shown in Fig. 6. In particular, the

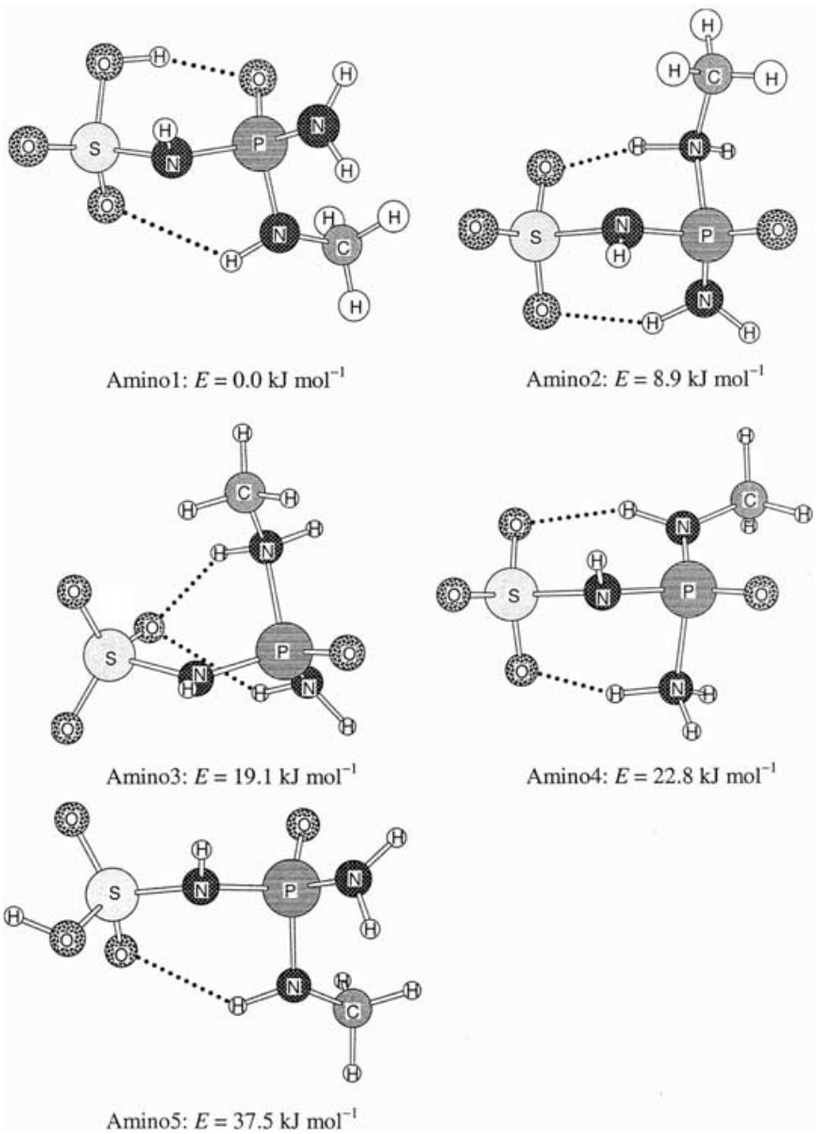

FIGURE 4 Amino tautomers of PSO. (Energies relative to amino1).

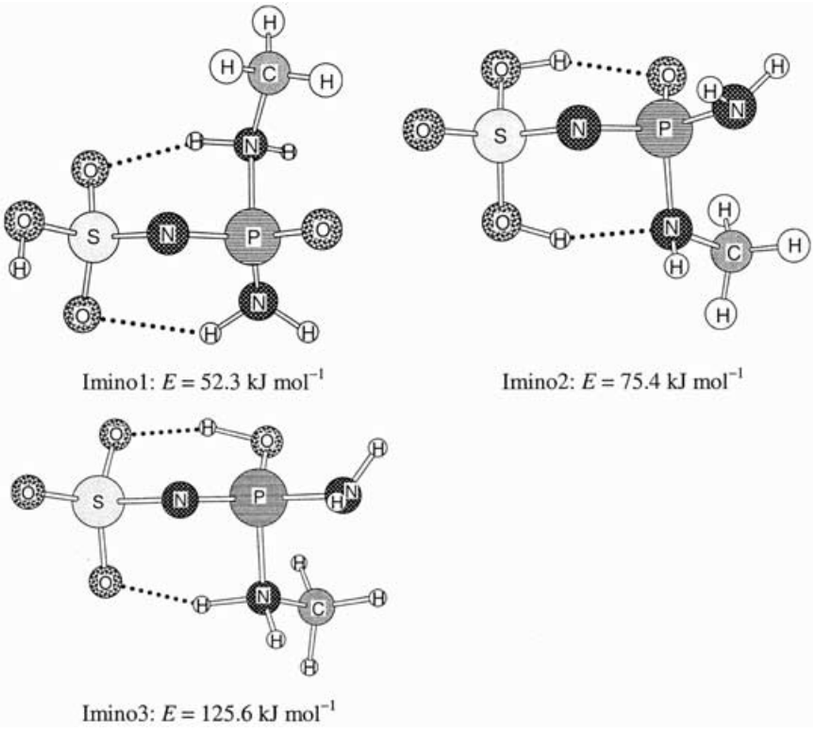

FIGURE 5 Imino tautomers of PSO. (Energies relative to amino1).

distance of 2.79 Å between the S–N–P nitrogen (N2) and the C–N–C nitrogen (N_{Arg}) of the arginine residue suggests a strong hydrogen bond mediated N···N interaction, as noted by Langley *et al.* [1]. However, the N2···N_{Arg} interaction may be expected to be destabilising in the case of an amino tautomer, since the near-planar arrangement of the S–N2–P and C–N_{Arg}–C groups would imply that the N2–H and N_{Arg}–H groups would be pointing towards each other, which would result in strong repulsion between the hydrogens. As this interaction is expected to have the most significant effect on the relative stabilities of the tautomers, it was studied in some detail. Two approaches were used: (a) full optimisation of the geometry (relaxed calculation) and (b) partial optimisation, where the heavy atom backbone is constrained at the X-ray geometry and only the hydrogen positions are optimised (unrelaxed calculation). The first approach has the advantage of yielding optimal geometries, including hydrogen bond distances, and interaction energies. However, the strong intramolecular hydrogen bonds in the inhibitor, as discussed in the previous section, could considerably

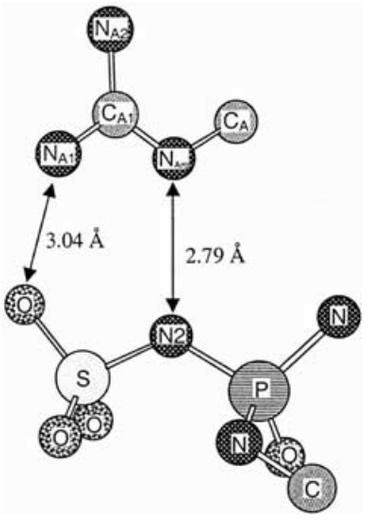

FIGURE 6 X-ray structure of PSOrn fragment with Arg57 residue.

deform the structure, thus making comparisons between the computed equilibrium geometries and the X-ray values effectively meaningless. (In reality the enzyme bound PSOrn forms intermolecular hydrogen bonds to the various residues around it, in preference to intramolecular hydrogen bonds.) To simplify the calculations only the interaction between PSO and a truncated form of the arginine residue ($C_2N_3H_7$), as shown in Fig. 6, was studied.

Initially, the range of PSO–arginine adducts studied were hydrogen bonded complexes of the various (amino and imino) tautomers of PSO (as shown in Figs. 4 and 5) and neutral arginine, i.e. dimers. The lowest energy dimer in this group is a complex involving the amino1 tautomer, with binding energies computed as 27.5 and 11.3 kJ mol^{-1} from the relaxed and unrelaxed calculations, respectively. This suggests that the N2$\cdots$N$_{Arg}$ interaction is stabilising, although the N2$\cdots$N$_{Arg}$ distance is considerably longer than in the X-ray structure. As can be seen from the structure in Fig. 7, in the complex the arginine moiety is distorted, with the N$_{Arg}$–H bond rotated out of the molecular plane. The interaction of the imino1 tautomer with arginine gives rise to considerably larger binding energies: 50.9 and 26.8 kJ mol^{-1} from the relaxed and unrelaxed calculations, respectively. Nevertheless, in absolute terms the amino1–arginine complex is more stable by $\sim$29 kJ mol^{-1}, as indicated by the relaxed calculations.

On extending the calculations to zwitterionic dimers, i.e. complexes of deprotonated PSO (denoted PSO$^-$) and protonated arginine, it was found that two

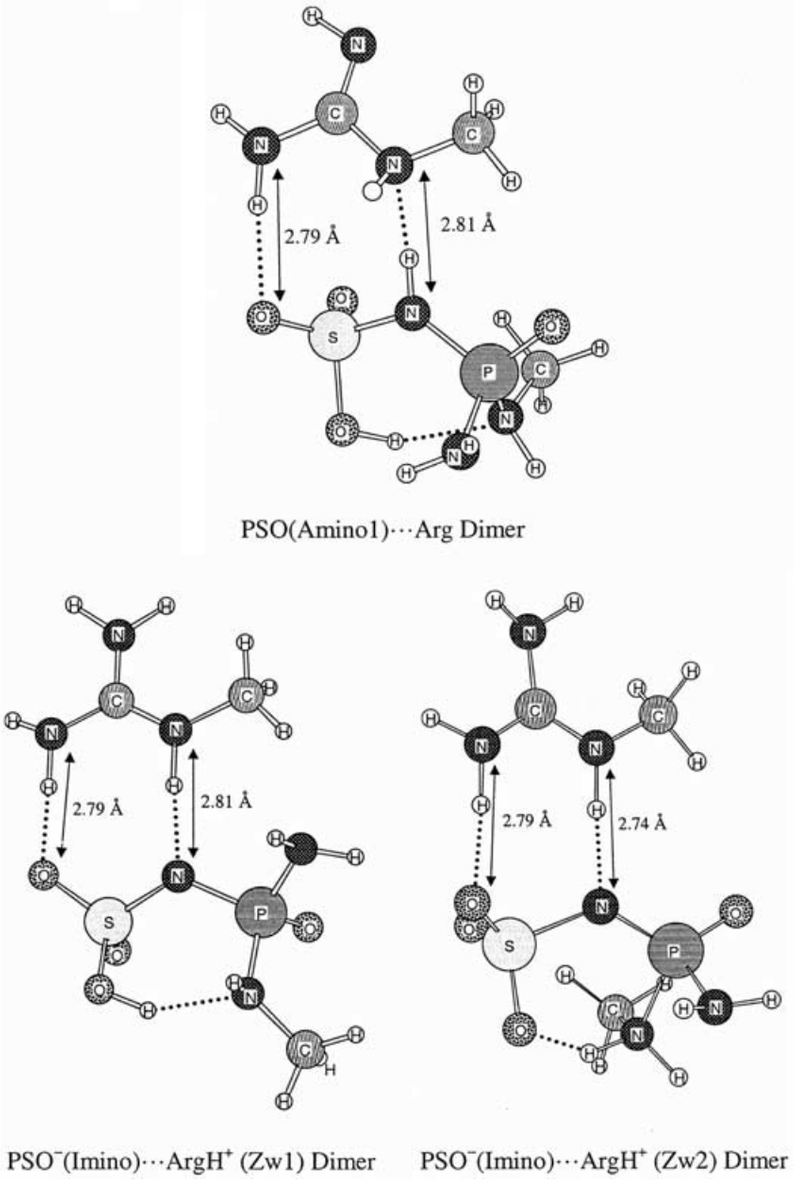

FIGURE 7 Structures of the three most stable PSO···Arg dimers.

of these are more stable, even in gas phase, than the dimers between neutral partners. The structures of these complexes (denoted Zw1 and Zw2) are also shown in Fig. 7. The PSO^- moieties in both of these dimers are imino tautomers. As can be seen from the tabulated distances in Table I, the fully optimised geometry of Zw1 matches the X-ray data reasonably well. Agreement between theory and experiment is less convincing in the case of Zw2, where the P–N1 bond distance of 1.89 Å is clearly at variance with the X-ray value of 1.61 Å. Zw2, however, appears to be the more stable (by 13.2 kJ mol^{-1}) of the two dimers. Compared with the lowest energy amino1–arginine complex, Zw1 and Zw2 were computed to be 24.0 and 37.2 kJ mol^{-1} more stable, respectively, corresponding to binding energies of 51.5 and 64.7 kJ mol^{-1} with respect to neutral arginine and the amino1 form of PSO. The stabilities of the various dimers, as well as of a trimer (as discussed below), are summarised in Table II as dissociation energies to a range of neutral and charged moieties.

TABLE I Selected X-ray distances for enzyme bound PSOrn and corresponding computed distances in PSO···Arg dimers and PSO···$(Arg)_2$ Trimer

	Atom–Atom distance (Å)			
	Bound PSOrn X-ray	PSO···Arg (Zw1)	PSO···Arg (Zw2)	PSO···$(Arg)_2$ Trimer
S–O	1.51	1.48	1.49	1.50
S–O1	1.34	1.63	1.50	1.52
S–O2	1.58	1.46	1.48	1.49
S–N2	1.60	1.62	1.69	1.65
N2–P	1.66	1.65	1.59	1.63
P–O3	1.48	1.49	1.49	1.52
P–N	1.72	1.71	1.69	1.71
P–N1	1.61	1.74	1.89	1.70
N1–C	1.48	1.47	1.49	1.47
N2–N_{Arg}	2.79	2.81	2.74	2.72
O–N_{A1}	3.04	2.76	2.76	2.70
C_A–N_{Arg}	1.46	1.46	1.45	1.45
N_{Arg} – C_{A1}	1.33	1.32	1.32	1.32
C_{A1} – N_{A2}	1.33	1.36	1.37	1.37
C_{A1}–N_{A1}	1.33	1.34	1.34	1.34
O1–N_{a1}	2.76			2.72
O3–N_{a2}	2.73			2.69
C_a–N_a	1.45			1.47
N_a–C_{a1}	1.33			1.36
C_{a1}–N_{a1}	1.32			1.34
C_{a1}–N_{a2}	1.32			1.34

Labelling of atoms as indicated in Fig. 1.
Subscripts Arg, A, A1, A2 refer to atoms of Arg57 (See Fig. 6).
Subscripts a, a1, a2 refer to atoms of Arg106.

TABLE II Computed dissociation energies of PSO···Arg dimers and trimers

	ΔE (kJ mol^{-1})
PSO (Amino1)···Arg Dimer → PSO(Amino1) + Arg	27.5
PSO (Imino1)···Arg Dimer → PSO(Amino1) + Arg	− 1.3
PSO (Imino1)···Arg Dimer → PSO (Imino1) + Arg	50.9
PSO^- (Imino)···$ArgH^+$ Dimer (Zw1) → PSO(Amino1) + Arg	51.5
PSO^- (Imino)···$ArgH^+$ Dimer (Zw1) → PSO^- (Imino) + $ArgH^+$	332.8
PSO^- (Imino)···$ArgH^+$ Dimer (Zw2) → PSO(Amino1) + Arg	64.7
PSO^- (Amino)···$ArgH^+$ Dimer (Zw3) → PSO(Amino1) + Arg	126.5
PSO^{2-}(Imino)···$(ArgH^+)_2$ Trimer → PSO(Amino1) + 2Arg	170.8
PSO^{2-}(Imino)···$(ArgH^+)_2$ Trimer → PSO^-···$ArgH^+$ Dimer(Zw1) + Arg	119.3
PSO^{2-}(Imino)···$(ArgH^+)_2$ Trimer → PSO^{2-} + 2$ArgH^+$	1284.2

The relative stabilities of PSO and PSO···Arg dimers, as obtained in constrained and relaxed calculations are shown in Fig. 8. The trends in the stabilities appear to be qualitatively reproduced by the constrained optimisations, but clearly the energy differences, especially between the PSO(Amino1)···Arg dimer and Zw1, are predicted to be considerably larger by the constrained calculations. As remarked in the previous section, in light of the large energy

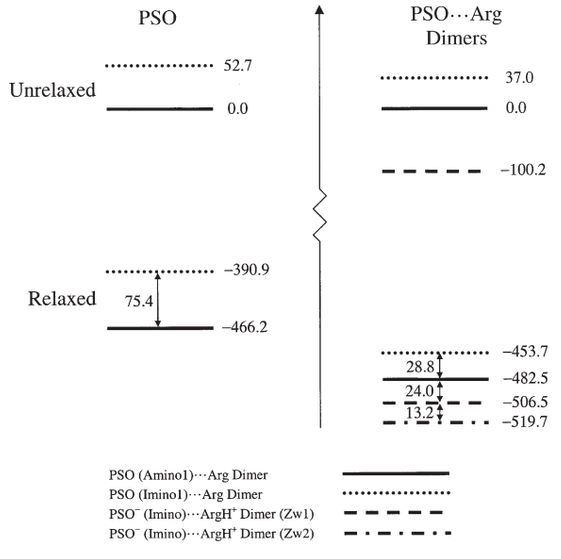

FIGURE 8 Relative energies (in kJ mol^{-1}) of PSO tautomers and PSO···Arg dimers from constrained and relaxed calculations.

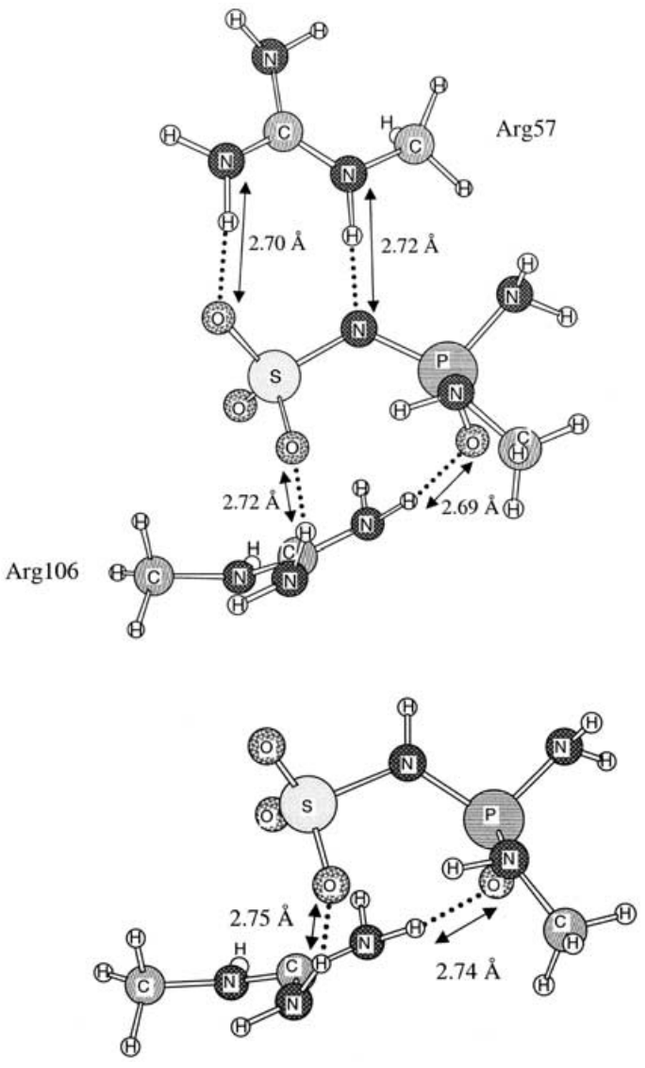

FIGURE 9 Structures of PSO···$(Arg)_2$ trimer and PSO···Arg (Zw3) dimer.

differences between the unrelaxed and relaxed structures, we regard the latter as the more reliable.

Interactions between amino tautomers of PSO^- and protonated arginine (denoted $ArgH^+$) were found to be repulsive, as expected. Although in the latter complexes, $ArgH^+$ did bind to PSO^-, this did not occur via N2, as would be required for a valid description of the binding of PSOrn in the enzyme.

According to the X-ray data PSOrn interacts with two arginine residues, the second (Arg106) effectively bridging the O1 and O3 atoms of PSOrn (see Fig. 1). A trimer of PSO with two arginines is clearly a more realistic model for the binding of PSOrn to the enzyme. Given the apparent propensity of arginine to exist in protonated form, our trimer calculations were restricted to a complex of a (doubly deprotonated) dinegative PSO (denoted PSO^{2-}) and two $ArgH^+$ subunits. The computed structure of this trimer is shown in Fig. 9. The key interatomic distances are listed in Table I. The agreement with the X-ray data is good, given the relatively high estimated errors of ± 0.2 Å in the X-ray distances. The large binding energy of $170.8\,kJ\,mol^{-1}$, relative to neutral PSO and two arginines, is consistent with the action of PSOrn as an effective inhibitior that binds irreversibly to the enzyme.

The very much higher stability of the trimer (to dissociation) than of the dimers Zw1 and Zw2 suggests that the interaction between a PSO^- ion and $ArgH^+$ (representing the Arg106 residue) is actually the dominant contribution to the overall stability, rather than the interaction with the (protonated) Arg57 residue. To test this hypothesis, the structure of a third zwitterionic dimer (Zw3) was optimised. This derives from the trimer by the removal of neutral Arg57 (See Fig. 9). PSO^- in Zw3 was chosen to be an amino tautomer. According to the calculations, Zw3 is more than twice as stable as Zw1, its dissociation energy to PSO and Arg having been computed to be $126.0\,kJ\,mol^{-1}$. Thus, the overall binding energy of the trimer, to a good approximation, is the sum of the individual binding energies to the two arginine residues.

In the zwitterionic dimers, as well as in the trimer discussed above, the overall binding between the negatively charged PSO^- and $ArgH^+$ moieties, in addition to the hydrogen bonding, has a substantial ionic (electrostatic) contribution. This can be quantified through the analysis of the binding energies of Zw1, Zw2 and the trimer, relative to neutral PSO and Arg as well as relative to the ions PSO^-, PSO^{2-} and $ArgH^+$. These results are included in Table II. Thus Zw1 and Zw2 are bound by nearly $350\,kJ\,mol^{-1}$ relative to the ions, but because of the very different proton affinities of PSO^- and Arg, the binding energies relative to the most stable amino tautomer of PSO and Arg are nearly an order of magnitude smaller. Furthermore, the $PSO^{2-}\cdots(ArgH^+)_2$ trimer, is bound by $1284\,kJ\,mol^{-1}$ relative to the ions.

Charge Distribution and Bonding

In Fig. 2, following the usual convention, the Lewis structures of amino and imino tautomers of PSOrn were drawn with several PO and SO double bonds, with the implication that P and S are hypervalent, viz. accomodate more than eight electrons in their valence shells. In the case of the P = O and S = O bonds this implies utilisation of the 3d atomic orbitals of P and S in the formation of the appropriate PO and SO π molecular orbitals. The validity of such "expansion of the octet" has been strongly debated in the literature over the past 20 years [4–7]. On the basis of careful quantum chemical studies, several authors have concluded that the electronic structures of molecules with apparently hypervalent multiply bonded second row atoms are best described by invoking semipolar bonds, where, e.g. the P and S atoms acquire formal charges of up to +2, which then allows these atoms to form up to four covalent bonds with O^- (or other atoms or ions) [4,5,7,8]. The semipolar bonds thus have both covalent and ionic components and in strength such bonds are comparable with double bonds with bond lengths to match.

In light of the above observations, the amino structure of PSOrn in Fig. 2 would be more correctly drawn by replacing each P = O and S = O with $P^+–O^-$ and $S^+–O^-$ semipolar bonds (which would result in S with a formal 2 + charge), as shown in Fig. 10. The imino tautomer, however, as drawn in Fig. 2, relies on π resonance to partially delocalise the negative charge on the PNS nitrogen due to the two lone pairs. Thus, if P and S cannot participate in π bonding, the above mechanism for charge delocalisation cannot be invoked. Hence our initial suspicion that amino forms of PSO would be considerably more stable than any imino tautomer. In the case of isolated PSO these suspicions proved well-founded, as the computations located five amino type tautomers which were more stable than the lowest energy imino tautomer (Figs. 4 and 5).

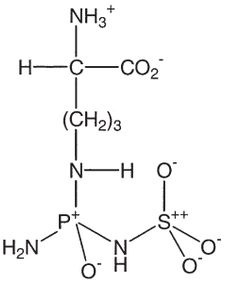

FIGURE 10 Lewis structure of an amino tautomer of PSOrn with semipolar bonds.

As in previous studies that addressed the problem of hypervalency, we have used the Roby–Davidson (RD) method [9–12] to carry out population analyses on free as well as bound PSO, yielding atomic charges and shared electron numbers (σ) for pairs of atoms. The latter is interpreted as a direct measure of the covalent character of a given bond. It must be noted, however, that shared electron numbers are not bond orders, i.e. their interpretation requires calibration. This was carried out by an analysing the shared electron numbers of a range of small molecules (H_2PNH_2, $PO(NH_2)_3$, H_3PNH, HPNH, HPO, HOPO, H_3PO, $HSNH_2$, H_2SNH, SNH, HSOH, H_2SO_4, H_3SO, SO) and correlating the shared electron numbers with bond lengths and bond orders, provided the latter could be reasonably assigned, e.g. double bonds in S = O and HP = O, and single bonds in $H_2P–NH_2$ and $HS–NH_2$. Thus, for PN and PO: $\sigma = 1.0–1.22$ are consistent with single bonds, $\sigma = 1.76–1.83$ describe double bonds, while $\sigma = 1.40–1.50$ apply to semipolar bonds. Lower σ values describe such bonds in the case of SN and SO linkages.

The computed shared electron numbers and atomic charges for the amino1 and imino1 tautomers of PSO, along with those of the zwitterionic dimer Zw1 are listed in Tables III and IV. Almost all the bonds between the heavy atoms of PSO are described as single or semipolar bonds. A partial double bond character has been assigned to the P–N2 bond in the iminol tautomer of PSO. In this molecule, due to the protonation of N1, the P–N1 bond is long and weak and, therefore, a degree of bonding π interaction between the P and N2 atoms is possible. The high positive charges on the S and P atoms along with the high negative charges on the oxygens of PSO are consistent with semipolar SO and PO bonds. The high negative charge on N2 is according to expectations in the case of imino tautomers, although it is quite high in the amino1 form as well, due to the polar

TABLE III Selected bond lengths (R inÅ), Roby–Davidson shared electron numbers (σ in e) and assigned bond types of amino and imino tautomers of PSO and of the PSO···Arg dimer (Zw1)

	PSO (Amino1)			PSO (Imino1)			PSO···Arg Dimer (Zw1)		
	R	σ	Bond Type	R	σ	Bond Type	R	σ	Bond Type
S − O	1.45	1.3	Semipolar	1.63*	0.8	Semipolar	1.48	1.1	Semipolar
S − O1	1.46	1.2	Semipolar	1.47	1.2	Semipolar	1.63*	0.8	Semipolar
S − O2	1.61*	0.8	Single	1.48	1.1	Single	1.46	1.2	Single
S − N2	1.70	0.9	Single	1.61	1.1	Part. Double	1.62	1.1	Single
P − N2	1.72	1.0	Single	1.60	1.3	Single	1.65	1.1	Single
P − O3	1.50	1.3	Semipolar	1.48	1.5	Semipolar	1.49	1.4	Semipolar
P − N	1.66	1.1	Single	1.67	1.1	Single	1.71	1.0	Single
P − N1	1.67	1.1	Single	1.92	0.6	Weak Single	1.74	0.9	Single

Labelling of atoms as indicated in Fig. 1.
* Part of SOH group.

TABLE IV Atomic Charges (in *e*) on heavy atoms for PSO in amino and imino tautomers of PSO and of the PSO···Arg dimer (Zw1) from Roby–Davidson analysis

	PSO (Amino1)	PSO (Imino1)	PSO···Arg dimer (Zw1)
S	1.79	1.77	1.84
O	−0.72	−0.46*	−0.82
O1	−0.59	−0.81	−0.59*
O2	−0.53*	−0.64	−0.74
N2	−0.82	−1.08	−1.19
P	1.32	1.27	1.05
O3	−0.89	−0.78	−0.76
N	−0.56	−0.62	−0.46
N1	−0.54	−0.28	−0.33

Labelling of atoms as indicated in Fig. 1.
*Part of SOH group.

NS, NP, but especially NH bonds. Interestingly, there is an increased negative charge localisation on N2 in the case of the Zw1 dimer. This is probably due to the polarisation of PSO by the positively charged arginine residue.

The population analyses for the amino1 and imino2 tautomers were repeated with basis sets containing two additional sets of d-functions on the P and S atoms (with exponents chosen as 1/3 and 1/9 of those in the 6-31G(d) sets). This was done to ensure that the description of the atomic 3d orbitals on these atoms is sufficiently accurate and flexible to resolve any incipient π bonding. No significant changes in charges, shared electron numbers or relative energies occurred. We concluded therefore that no appreciable π bonding is present in the various tautomers of PSO and its complexes.

CONCLUSION

With the aid of quantum chemistry, viz. density functional theory, the binding of PSOrn to the enzyme OTCase was investigated and modelled through a study of PSO, a simplified model for PSOrn, and its interaction with one and two arginine molecules. The PSO···$(Arg)_2$ trimer was found to be bound by $\sim 171\,kJ\,mol^{-1}$. Such high stability, due to the presence of four hydrogen bonds as well as a large ionic interaction between the dinegative PSO^{2-} and protonated arginines, is consistent with the experimental observation that PSOrn is a powerful enzyme inhibitor. The calculations confirm the proposals of Langley *et al.*, [1] in as much as bound PSOrn is a dinegative imino tautomer. While in the case of free (neutral) PSO the most stable tautomers were calculated to be amino types, when bound to one or two (protonated) arginines PSO (as PSO^{-} or PSO^{2-}) is predicted to prefer an imino form. However, as in other phosphorous and sulfur containing

molecules, according to the population analyses that were carried out, the PN, PO, SN and SO bonds in PSO are generally best described as single or semipolar bonds.

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