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A Computational and Conceptual DFT Study of the Reactivity of Anionic Compounds: Implications for Enzymatic Catalysis

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We present a DFT study on the reactivity of an alkoxide and a thiolate toward the neutral and all ionic forms of arsenate and phosphate. The isolated neutral, mono-, di-, and trianionic arsenate/phosphate (electrophiles) and thiolate/methanolate (nucleophiles) species are used as model systems of reacting partners in the enzymatic reactions catalyzed by arsenate reductase and phosphatase. The onset of the displacement reaction at the electrophilic center by the attacking nucleophile is described by the DFT descriptor local softness, applied in a HSAB context. The instability of multiply charged anions in gas phase necessitates the use of a solvent model with an appropriate dielectric constant mimicking the enzymatic environment. Gas-phase and solvent-reactivity studies of isolated compounds indicate that the nucleophilic attack of a thiolate during the first catalysis step of arsenate reductase (ArsC) and low molecular weight phosphatase (LMWPTPase) preferably occurs via the dianionic arsenate and phosphate. This computational approach confirms and supplements earlier experimental data.

1. Introduction

In the present work, isolated arsenate, phosphate, and thiolate are considered as model systems for the enzymatic catalyzed nucleophilic reaction of the related enzymes arsenate reductase (ArsC) of *Staphylococcus aureus* and low molecular weight phosphatase (LMWPTPase). In their first reaction step, these enzymes catalyze the nucleophilic attack of a thiolate on arsenate[1] or on phosphate,[2] respectively (Figure 1). The pH optimum for enzymatic catalysis lies in the range 6.3−8.0 for ArsC[3,4] and 5−8 for LMWPTPase.[5] Since these pH ranges include the pK_{a2} values of arsenate (6.9) and phosphate (7.2), determination of the protonation state of the substrate remains problematic.

In the past, both experimental studies (kinetic isotope investigations and pH rate profiles) and computational investigations (pK_a calculations and mechanistic studies with calculation of reaction energies) have been conducted for PTPases,[2,6−11] leading to proposals of either a monoanionic[7,8,10] or a dianionic[2,6,9,11] substrate. The proposed thiolate attack on a dianionic substrate experiences resistance because of the large electrostatic repulsion between the dianionic substrate and the negatively charged nucleophile in the active site.[7] Recently, however, the dianionic substrate received strong support from a density functional theory (DFT) study on the mechanism of tyrosine PTPase.[12]

In the case of ArsC, the situation is even poorer: kinetic isotope investigations and pH rate profiles cannot be performed because of the instability of arsenate esters.[13] This means that the protonation state of arsenate in the active site of ArsC is experimentally not accessible. Since the knowledge of the protonation state of enzyme-bound substrates is indispensable for the understanding of enzymatic catalysis, theoretical studies affording its prediction are of crucial importance. From this point of view, in this study, the strong indication for a dianionic bound phosphate in LMWPTPase will be investigated. Also, the question concerning the protonation state of the enzyme-bound arsenate in ArsC will be addressed.

This study will be performed by considering the reactivity between the following isolated systems: $H_3AsO_4/H_2AsO_4^-/HAsO_4^{2-}/AsO_4^{3-}$ and $H_3PO_4/ H_2PO_4^-/HPO_4^{2-}/PO_4^{3-}$ as electrophiles and isolated CH_3S^- as a nucleophile in all possible combinations of electrophiles and nucleophiles. Since arsenic and phosphorus are chemically related, it is also interesting to compare the nucleophile CH_3S^- with the chemically related CH_3O^-. As such, the relative reactivities of both electronically related nucleophiles and electrophiles can be compared.

Formally, the following displacement reactions that could be considered as a measure of the reactivity of the substrate to the nucleophile can be written for these nucleophiles and electrophiles:

$$CH_3X^- + H_3YO_4 \rightarrow CH_3X{-}YH_2O_3 + OH^-$$

$$CH_3X^- + H_2YO_4^- \rightarrow CH_3X{-}YHO_3^- + OH^-$$

$$CH_3X^- + HYO_4^{2-} \rightarrow CH_3X{-}YO_3^{2-} + OH^-$$

$$CH_3X^- + YO_4^{3-} \rightarrow CH_3X{-}YO_3^{3-} + O^-$$

with X sulfur or oxygen and with Y arsenic or phosphorus.

In the gas phase, one is confronted with the problem of the instability of multiply charged anionic compounds with respect to electron emission because of strong Coulomb repulsion.[14]

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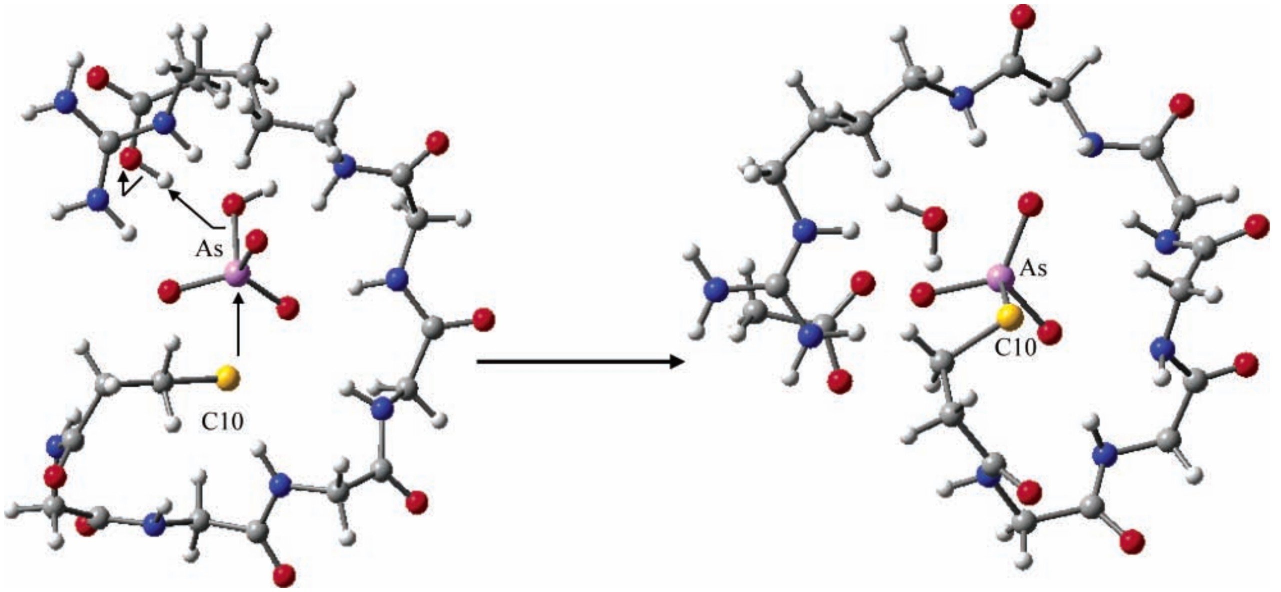

Figure 1. Schematic representation of the active site of ArsC (LMWPTPase) and the enzyme-catalyzed nucleophilic attack of a thiolate (here Cysteine 10 (C10)) on arsenate, with formation of a covalent sulfer-arseno intermediate. Color code: O (red), As (purple), N (blue), S (yellow), C (gray), H (white).

This instability is seen by the positive HOMO energies, obtained in agreement with ref 15 for the di- and trianionic species in the gas-phase. However, tunneling through a barrier of several eV is necessary for these anions to detach an electron,[16] indicating their metastability. Since these anions play an important role in chemistry and biochemistry, where they are stabilized in solutions and in enzymatic systems, we will also use a solvent model, where the dielectric constant is a scaling parameter meant to represent contributions of the enzymatic environment that are not included explicitly in the model.[17]

This reactivity analysis is adequately performed within a conceptual density functional theory (DFT) context, using the hard and soft acids and basis (HSAB) principle, at a local level. Since both reagents (As/P and O/S) are highly polarizable and thus soft[18] and since the displacement reactions are most likely orbital controlled, a crucial role will thus be played by the softness.[19] The softness matching procedure as proposed by the present authors[20] (for a generalization see Ponti[21]) is then a useful strategy to test all possible combinations of the reagents in a local/local approach.[22]

In recent years, there has been an increasing interest in density functional theory, both in its conceptual and computational aspects.[23−27] Using the electron density as the fundamental property leads to a better quality/cost ratio when evaluating molecular properties (computational DFT). On the other hand, conceptual DFT[27−30] offers many concepts to describe the reactivity between reaction partners. Global reactivity descriptors of this type[23,30] are electronegativity, chemical potential, global hardness, and global softness. Local hardness, local softness, and Fukui functions are examples of local reactivity descriptors.[23,30] A quantitative treatment was hampered for a long time because of a shortage for quantifying hardness and softness. A breakthrough was reached in Parr and Pearson's seminal work[31] identifying the chemical hardness as the difference between the ionization energy (I) and the electron affinity (A) of a species. Both the experimental determination and the quantum chemical evaluation of these properties were thereby made possible. Within the same context of the conceptual DFT, Parr et al.[32] presented evidence for the HSAB principle, and a more detailed treatment was presented later by Gázquez and Méndez.[33]

In the past, the concepts of hardness and softness and their local counterparts were used in an abundance of reactivity studies, considering almost all neutral or monoanionic systems.[30] In this study, we will analyze the global and local softness of neutral, mono-, as well as dianionic and trianionic species of arsenate and phosphate and their consequence for interaction on the basis of the HSAB principle.

2. Theory and Computational Details

The DFT descriptor used in this work is the local softness $s(\mathbf{r})$, obtained as

$$s(\mathbf{r}) = f(\mathbf{r})S \tag{1}$$

where S is the global softness of the system[34] and $f(\mathbf{r})$ is the so-called Fukui function,[35] a frontier MO reactivity index defined as the derivative of the electronic chemical potential μ with respect to the external (i.e., because of the nuclei) potential $\nu(\mathbf{r})$. This can also be written as the change of the electron density in case an electron is withdrawn or added to the system.

$$f(\mathbf{r}) = \left[\frac{\delta\mu}{\delta\nu(\mathbf{r})}\right]_N = \left[\frac{\partial\rho(\mathbf{r})}{\partial N}\right]_{\nu(\mathbf{r})} \tag{2}$$

As $\rho(\mathbf{r})$ is expected to be a discontinuous function of the number of electrons N, the use of different reactivity descriptors was proposed for electrophilic and nucleophilic attacks.

The left derivative can be used when an electrophilic attack is to be studied

$$f^{-}(\mathbf{r}) = \left[\frac{\partial\rho(\mathbf{r})}{\partial N}\right]^{-}_{\nu(\mathbf{r})} \tag{3}$$

for the attack of a nucleophile the right derivative is to be chosen

$$f^{+}(\mathbf{r}) = \left[\frac{\partial\rho(\mathbf{r})}{\partial N}\right]^{+}_{\nu(\mathbf{r})} \tag{4}$$

Within a finite difference approximation combined with the idea of integrating the Fukui function over atomic regions, one finds the condensed Fukui functions for nucleophilic and electrophilic attack on atom A having N electrons:

$$f^{-}_{\mathrm{A}} = q_{\mathrm{A}}(N_0) - q_{\mathrm{A}}(N_0 - 1) \tag{5}$$

$$f^{+}_{\mathrm{A}} = q_{\mathrm{A}}(N_0 + 1) - q_{\mathrm{A}}(N_0) \tag{6}$$

$q_{\mathrm{A}}(N_0)$, $q_{\mathrm{A}}(N_0 + 1)$, and $q_{\mathrm{A}}(N_{0\,-}\,1)$ are the atomic populations for atom A in the neutral molecule (N_0 electrons) and the corresponding anion ($N_0 + 1$) or cation ($N_0 - 1$), all evaluated at the neutral (as reference) molecular geometry (cf. the demand for constant extern potential in eq 2).

For calculating the condensed Fukui function f_{A}, we will use NPA charges[36] calculated on the fully optimized (using the DFT-B3LYP methodology[37] with a 6-31+G** basis set[38]) structures of arsenate/phosphate and thiolate/methanolate described in the Introduction.

We select NPA charges for the calculation of Fukui function indices on the basis of several successful applications in the past of these population analysis in the calculation of Fukui function and local softness of neutral and monoanionic systems.[39] This population analysis offers the important advantage that we obtain in contradiction to Mulliken population analysis and electrostatic potential derived charges (ChelpG) non-negative Fukui function indices for a series of dianionic systems (arsenate, phosphate, sulfate, and selenate) and for the trianions AsO_4^{3-} and PO_4^{3-} as well. This is important in view of the fact that although the problem of negative Fukui function indices has been looked upon in detail,[40] no definitive answer has been given yet to the question whether negative values are physically acceptable or are artifacts.

As a direct consequence of eqs 3 and 4, two types of local softness are defined:

$$s^{-}(\mathbf{r}) = Sf^{-}(\mathbf{r}) \tag{7}$$

$$s^{+}(\mathbf{r}) = Sf^{+}(\mathbf{r}) \tag{8}$$

with associated condensed local softness

$$s_{\mathrm{A}}^{\;-} = Sf_{\mathrm{A}}^{\;-} \tag{9}$$

$$s_{\mathrm{A}}^{\;+} = Sf_{\mathrm{A}}^{\;+} \tag{10}$$

In our study, s^{+} indicates the local softness of arsenic/phosphorus and s^{-} the local softness of sulfur/oxygen.

The global softness S is given by the finite difference approximation

$$S = \frac{1}{I - A} \tag{11}$$

where I and A are the ionization energy and the electronaffinity, respectively.

The concepts of hardness and softness of a system had already been introduced by Pearson in the 1960s. They were at that time used in the explanation of acid−base reactions in their most general form (Lewis acid/Lewis base).

Pearson then stated that "Hard Acids prefer to react with Hard Bases whereas Soft Acids prefer to interact with Soft Bases." This is known as the hard and soft acids and bases (HSAB) principle.

According to this principle and in analogy with earlier work by Gázquez[41] and our group[42] and its generalization by Ponti,[21] the (preferred) reactivity between the reaction partners can be based on the difference in local softness *s(r)* of the interacting parts (atoms, functional groups, ...) of these reaction partners

$$\Delta \mathrm{s}(r) = |\mathrm{s}^{+}(r) - \mathrm{s}^{-}(r)| \tag{12}$$

which should be minimal for optimal interaction, a criterion used throughout this paper.

The HSAB principle offers the advantage that the characteristics of a reaction (mainly kinetic aspects) are described in terms of the properties of the reagents (in this particular case called reagents and substrate), in the ground state, without explicit numerical calculation of characteristics along the reaction path. The use of the HSAB principle is indeed based on a perturbational "ansatz" as formulated by Parr.[23] Assuming that reaction paths will not cross (Klopman's rule[43]), the relative energies at the beginning of the reaction obtained in this approach thus can be expected to predict a sequence of activation energies from information on the reactant properties only.

When a solvent model is used, the solvent is treated as a continuum with a uniform dielectric constant surrounding the isolated arsenate and phosphate as solute molecules which are placed in a cavity (continuum method[44]). As a method for defining the cavity and the reaction field, the self-consistent isodensity polarized continuum model (SCI−PCM[45(a−c)]) is chosen[42d] with an isodensity surface specified as 0.001 au.[45d]

All structures (coordinates given in the Supporting Information) were optimized in gas phase and solution with the dielectric constants used, before the properties were calculated.

All calculations were performed in a DFT context using the B3LYP exchange correlation functional[37] with a 6-31+G** basis set[38] using the GAUSSIAN 98[46] package.

3. Results and Discussion

Calculation of S. The computation of the global softness for the di- and trianionic species in gas-phase with eq 11 is problematic because of the obvious rise of the E versus N curve caused by their instabilty in the gas-phase. The unusual evolution of this curve is also found in the prototypical multiply charged gas-phase unstable sulfate anion.[47] To overcome this problem, one can apply Koopman's theorem[48] (assumption of frozen orbitals) as an alternative approach, approximating I by the energy of the highest occupied molecular orbital (ϵ_{HOMO}) and A by the energy of the lowest unoccupied molecular orbital (ϵ_{LUMO}), yielding for S:

$$\mathrm{S} = 1/(\epsilon_{\mathrm{LUMO}} - \epsilon_{\mathrm{HOMO}}) \tag{13}$$

Before adopting eq 13 as a working equation in the calculation of S, a remark should be made. Koopman's theorem is defined within the context of Hartree−Fock theory. DFT identifies I with the HOMO energy[49] but does not make a statement about A, making the proposed methodology less well established. Calculating the properties with Hartree−Fock theory does not pose an alternative either, because in applying this method the inclusion of electron correlation is missing. However, the observation that the hardness (inverse of the softness) correlates with the band gap,[50] the difference between the LUMO and HOMO energies, advocates for using eq 13 as working equation.

To further assess our adopted methodology, we use the well-established proportionality between the global softness and the polarizability α of a system, presented and used several times in the literature,[51] for systems with spherical symmetry such as atoms and monatomic ions.

As in a first approximation the neutral and deprotonated forms of arsenate and phosphate are also highly symmetrical systems,

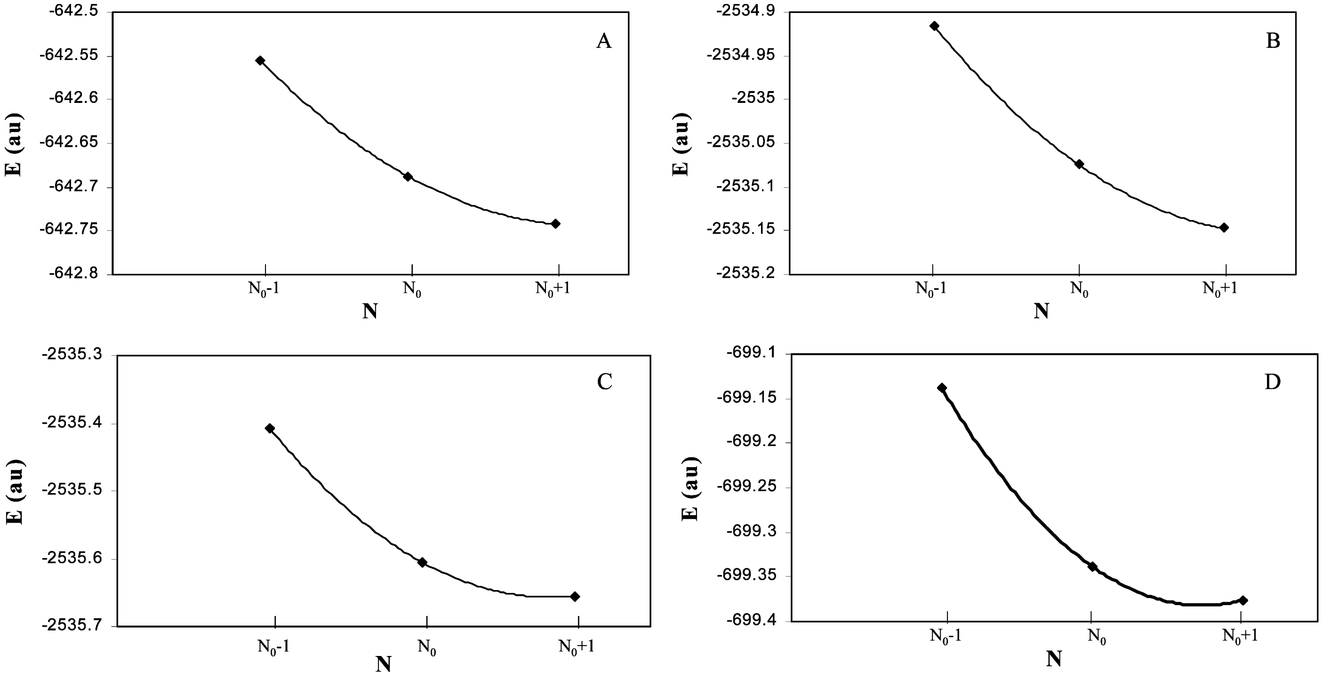

Figure 2. Energy (E) versus number of electrons (N) curve for (A) PO_4^{3-}, (B) AsO_4^{3-}, (C) $HAsO_4^{2-}$, and (D) SO_4^{2-} in solvent ($\epsilon = 20.7$). SO_4^{2-}, a frequently studied multiply charged anion, is included for comparison. N_0 always refers to the number of electrons of the reference anion.

we can consider the correlation between the calculated S and α for the series $H_3AsO_4/H_2AsO_4^-/HAsO_4^{2-}/AsO_4^{3-}/$ $H_3PO_4/H_2PO_4^-/HPO_4^{2-}/PO_4^{3-}$, where α is now calculated as the arithmetic average of the three diagonal elements of the polarizability tensor

$$\alpha = (\alpha_{xx} + \alpha_{yy} + \alpha_{zz})/3 \tag{14}$$

The situation is quite different in solvent. It is well known from experimental data that multiply charged anions become stabilized in solvent. When the solvent effect is approximated by a continuum model, the multiply charged anions considered are also stabilized (moreover, negative HOMO energies as well as declining E versus N curves are encountered (Figure 2)). So, we are in a position to calculate the global softness for these species with eq 11. This allows a comparison of the correlation between S and α, found when S is calculated by eq 11 (finite difference method) or by eq 13 (HOMO−LUMO gap) for the series $H_3AsO_4/H_2AsO_4^-/HAsO_4^{2-}/AsO_4^{3-}/H_3PO_4/H_2PO_4^-/HPO_4^{2-}/PO_4^{3-}$ stabilized in solutions with different dielectric constants. ϵ values of 20.7 and 78.39 were chosen motivated as follows: 20.7 is the ϵ value that we will use later in this study to mimic the enzymatic environment and 78.39 is the ϵ value of water.

Clearly, eqs 11 and 13 are never used simultaneously for studying a sequence of reactivity. They are only used to compare the adequateness of the approximation eq 13 to the "exact" (finite difference) eq 11.

The results of this comparison indicate that both methods to calculate S—finite difference as well as HOMO/LUMO gap—give almost the same, high correlation between S and α (Figure 3 and Tables 1−4).

Politzer was the first to put forward a relationship between the polarizability and the softness.[51a] Vela and Gázquez derived an expression of the proportionality between S and α.[51b] Correlations between S and $\alpha^{1/3}$ on the other hand were presented by various other authors.[52]

Here, both proportionalities are tested yielding correlation coefficients of comparable magnitude (Table 4).

The equivalence of the two methods (eqs 11 and 13) as well as the comparable values of R^2 found in gas phase and in the solvent models give confidence for the calculation of S by eq 13, even in a DFT approach. Consequently, in this work, we will use eq 13 throughout.

Gas-Phase Model. On the basis of the sequence of increasing polarizability while descending a column in the periodical system, one should expect arsenic to be softer than phosphorus and sulfur softer than oxygen (confirmed in the overview table given by Parr[23]).

The global softness of CH_3S^- is higher than that of CH_3O^- and as we notice, the softness of the sulfur atom remains higher than that of the oxygen atom (Table 5), which is in line with the higher softness of the isolated sulfur atom as compared to the oxygen atom.[53]

The global softness of arsenates is higher than that of phosphates with the exception of the trianionic compounds. In the neutral molecule, phosphorus is 0.07 au^{-1} softer than arsenic. In the di- and monoprotonated species, arsenic is softer than phosphorus, but when the trianion is considered, phosphorus becomes much softer than arsenic (Table 1), which is the opposite of the expectation based on the literature.

Confronting the local softness of arsenic and phosphorus with sulfur and oxygen (in all possible combinations) gives the following sequences of reactivity (applying the HSAB principle (eq 12) (Table 6).

neutral: P−O > As−O > P−S > As−S

monoanion: As−O > P−O > As−S > P−S

dianion: As−S > P−O > P−S > As−O

trianion: As−S > As−O > P−S > P−O

Experimental data indicate that in phosphate esters an alkoxide is a much better nucleophile than a thiolate.[54] Our calculations of reactivities between phosphorus and oxygen/sulfur for the phosphoesters are consistent with these experimental data.

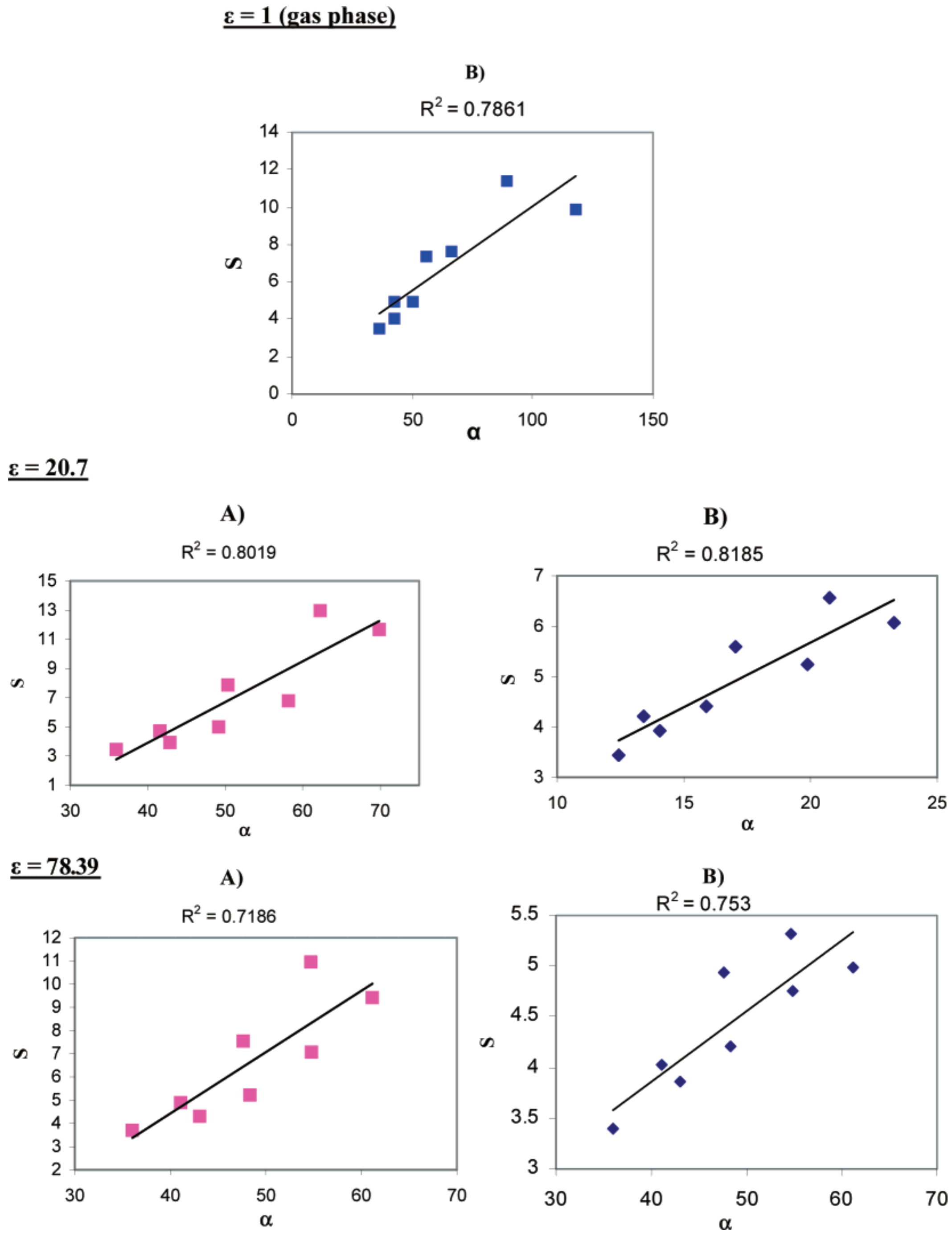

Figure 3. Correlation between global softness (S) calculated by eq 11 (A) and eq 13 (B) and polarizability (α) calculated by eq 14 for the species considered in this work: $H_3AsO_4/H_2AsO_4^-/HAsO_4^{2-}/AsO_4^{3-}/H_3PO_4/H_2PO_4^-/HPO_4^{2-}/PO_4^{3-}$. (Data from tables 1−3.)

The greatest reactivity between sulfur and phosphorus is found for the dianionic phosphate (Table 6), which is in line with the conclusions drawn from experimental and earlier theoretical studies,[2,6,9,11,12] the results of which strongly endorse the nucleophilic attack on a dianionic rather than a monoanionic substrate in the reaction catalyzed by LMWPTPase. These results obtained when applying the HSAB principle to isolated compounds in the gas phase may be directing when studying enzymatic reaction mechanisms.

At first sight, the increased reactivity toward sulfur in the series $H_3AsO_4 < H_2AsO_4^- < HAsO_4^{2-} < AsO_4^{3-}$ (Table 6) complies with the increasing values of the k_{cat}[3] with increasing pH of the reductase reaction as catalyzed by ArsC. However, the measured k_{cat} values are macroscopic rate constants. This means that the comparison between these experimental data and

TABLE 1: Softness and Polarizability of the Electrophiles in Gas Phase[a]

	S (eq 13)	α	s^+
H_3AsO_4	4.029	42.90	1.982
$H_2AsO_4^-$	4.914	50.51	3.561
$HAsO_4^{2-}$	7.648	66.54	6.438
AsO_4^{3-}	9.905	117.65	6.821
H_3PO_4	3.500	35.94	1.989
$H_2PO_4^-$	4.920	42.40	3.395
HPO_4^{2-}	7.364	55.63	5.424
PO_4^{3-}	11.388	89.23	10.771

[a] Global softness (S) (calculated from eq 13) and polarizability (α) (calculated from eq 14) of arsenate and phosphate and local softness (s^+) (calculated from eq 10) of the electrophilic arsenic (As) and phosphorus (P) atom (in a.u.). ($\epsilon = 1$.)

TABLE 2: Softness and Polarizability of the Electrophiles in the Enzymatic Environment ($\epsilon = 20.7$)[a]

	S (eq 11)	S (eq 13)	α	s^+
H_3AsO_4	3.933	3.923	42.87	1.855
$H_2AsO_4^-$	4.991	4.410	49.13	2.515
$HAsO_4^{2-}$	6.774	5.238	58.16	3.702
AsO_4^{3-}	11.675	6.064	69.88	5.079
H_3PO_4	3.450	3.439	35.92	1.748
$H_2PO_4^-$	4.713	4.209	41.59	2.172
HPO_4^{2-}	7.868	5.592	50.35	3.530
PO_4^{3-}	12.955	6.563	62.26	5.610

[a] Global softness (S) (calculated from eqs 11 and 13) and polarizability (α) (calculated from eq 14) of arsenate and phosphate and local softness (s^+) (calculated from eq 10) of the electrophilic arsenic (As) and phosphorus (P) atom (in a.u.). ($\epsilon = 20.7$.)

TABLE 3: Softness and Polarizability of the Electrophiles in Water ($\epsilon = 78.9$)[a]

	S (eq 11)	S (eq 13)	α
H_3AsO_4	4.306	3.855	43.07
$H_2AsO_4^-$	5.218	4.214	48.33
$HAsO_4^{2-}$	7.072	4.749	54.77
AsO_4^{3-}	9.424	4.995	61.16
H_3PO_4	3.696	3.402	36.03
$H_2PO_4^-$	4.894	4.019	41.09
HPO_4^{2-}	7.549	4.935	47.64
PO_4^{3-}	10.954	5.324	54.72

[a] Global softness (S) (calculated from eq 11 and 13) and polarizability (α) (calculated from eq 14) of arsenate and phosphate (in a.u.). ($\epsilon = 78.9$.)

TABLE 4: Values of the Correlation Coefficients R^2 for the Correlation between S, α, and $\alpha^{1/3}$ for Varying Dielectric Constants ϵ and for Varying Methods of Calculation of S

		correlation coefficients	
ϵ	S calculated by eq	α	$\alpha^{1/3}$
1	13	0.7861	0.8444
20.7	11	0.8019	0.7923
	13	0.8185	0.8351
78.9	11	0.7186	0.7162
	13	0.7530	0.7722

TABLE 5: Softness of the Nucleophiles in Gas Phase[a]

	S	s^-
CH_3S^-	7.963	6.807
CH_3O^-	7.257	4.821

[a] Global softness (S) of CH_3S^- and CH_3O^- (calculated from eq 13) and local softness (s^-) (calculated from eq 9) of the nucleophilic atoms sulfur (S) and oxygen (O) (in a.u.). ($\epsilon = 1$.)

TABLE 6: Reactivity in Gas Phase as Measured by Difference in Local Softness[a]

Δs (a.u.)	H_3AsO_4	$H_2AsO_4^-$	$HAsO_4^{2-}$	AsO_4^{3-}
CH_3O^-	2.838	1.259	1.617	2.000
CH_3S^-	4.824	3.246	0.369	0.014
	H_3PO_4	$H_2PO_4^-$	HPO_4^{2-}	PO_4^{3-}
CH_3O^-	2.831	1.425	0.603	5.950
CH_3S^-	4.818	3.412	1.383	3.964

[a] Differences in local softness, Δs, between As and O, As and S, P and O, and P and S calculated from eq 12. ($\epsilon = 1$.)

our theoretical calculations is only meaningful in the hypothesis that the values of the macroscopic k_{cat} reflect the rate of the first reaction step during the enzymatic catalysis. Nevertheless, these HSAB results are presented as a microscopic tool which may shed some light on the macroscopic data, in the sense that the agreement could mean that the nucleophilic attack could be the rate-limiting step of the enzyme-catalyzed reaction. From the calculated data, it is clear that according to the HSAB principle the nucleophilic attack of a thiolate on a dianionic substrate is favored over the attack on a monoanionic substrate during the first reaction step of the enzymatic catalysis by ArsC. In light of the results obtained with the HSAB principle for the description of the reactivity for LMWPTPase, we can argue on the same basis for the attack on a dianionic substrate in the case of ArsC. We finally note that although the greatest reactivity occurs for AsO_4^{3-}, considering this anion as a ligand is less relevant because if the pH reaches values over 11.53, where this anion becomes predominant, the three-dimensional structure of ArsC is lost.[3]

Because of the intrinsic pK_a values of the entities carrying electrophile and nucleophile centers, not all the displacement reactions presented in the Introduction are experimentally accessible. We therefore cannot compare the complete calculated reactivity sequence with experimental data.

Nevertheless, the values of our theoretical calculations of properties and reactivity that do not have an experimental counterpart are presented as they afford the examination of factors influencing reactivity over a broader range.

It can be seen that when the protonation state of the compounds decreases, both the global softness of the system and the local softness of the central atom of the species increase (Table 1). If the softness of the central electrophilic atoms As or P increases, the reactivity toward the softer sulfur atom (except for PO_4^{3-}) increases too, even taking the upper hand over the reactivity of the oxygen atom (except for HPO_4^{2-}). The decrease of reactivity of the electrophiles toward the harder oxygen atom is less pronounced when the electrophiles become softer (Table 6).

The reactivity sequence illustrates the evolution of the combination with the highest reactivity of the hard−hard combination (P and O), for the neutral molecule to the soft−soft (As and S) combination, for the trianionic compounds. The combination with the lowest reactivity goes from the soft−soft combination (As and S) in the neutral molecule to the hard−hard combination (P and O) in the trianions.

Another way to confirm the preference for interaction comes from the calculation of the reaction energies of the displacement reactions considered in this study.

We calculated the reaction energies (Table 7) for the nucleophilic attack on the mono- and dianionic substrates, which gives the following sequence of interaction strength:

$$\text{monoanion: As-O} > \text{P-O} > \text{As-S} > \text{P-S}$$

$$\text{dianion: As-O} > \text{P-O} > \text{As-S} > \text{P-S}$$

For the monoanionic substrates, the sequence is the same as those obtained by applying the HSAB principle. This is not the case for the dianionic substrates, for which the metastability of these compounds should be kept in mind. The observation that the HSAB principle seems already not to work for the dianionic compounds questions the use of the HSAB principle for trianionic substrates in gas phase. To investigate the role of the instability in the discrepancy between the reactivity sequences obtained by the application of the HSAB principle or by the calculation of the interaction energy, we therefore used a solvent model in which the considered anions are stabilized. Such a model also enables us to validate the conclusions drawn in gas-phase about the preferential protonation state of the substrate

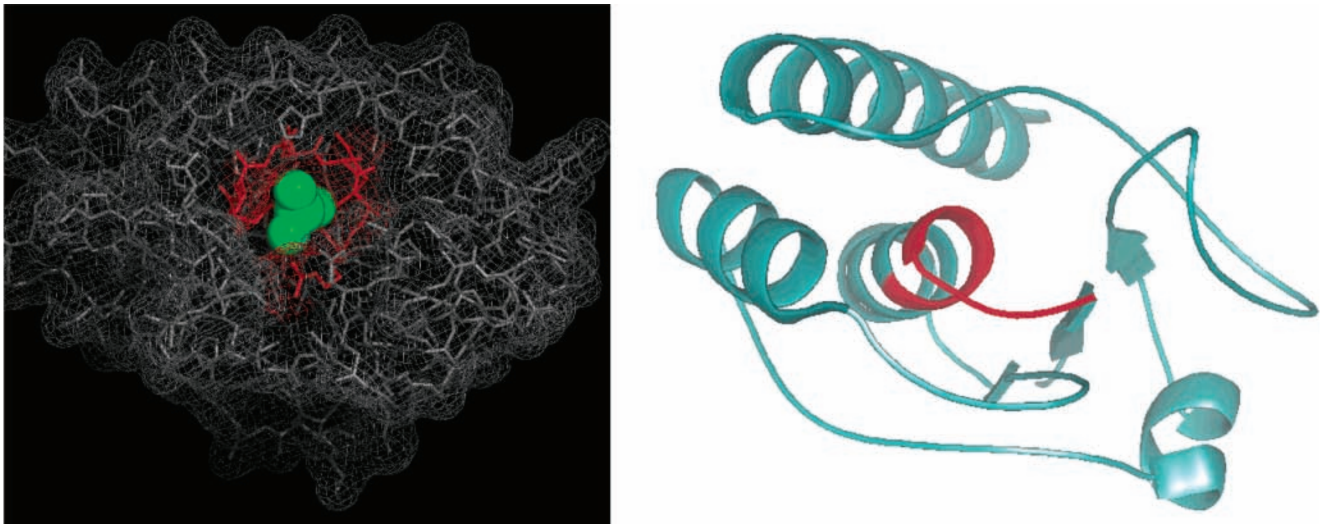

Figure 4. Solvent accessibility of the substrate binding pocket of ArsC. 3D-structure of ArsC. Red: substrate binding pocket; Green: substrate; Gray and blue: ArsC.

TABLE 7: Reaction Energies of Some Displacement Reactions (kcal/mol)[a]

	$\Delta E(\epsilon = 1)$	$\Delta E(\epsilon = 20.7)$
$CH_3S^- + HAsO_4^{2-}$	22.22	13.59
$CH_3S^- + HPO_4^{2-}$	24.53	14.10
$CH_3O^- + HAsO_4^{2-}$	4.18	−0.12
$CH_3O^- + HPO_4^{2-}$	4.93	−4.63
$CH_3S^- + H_2AsO_4^-$	30.96	7.84
$CH_3S^- + H_2PO_4^-$	37.64	15.24
$CH_3O^- + H_2AsO_4^-$	5.91	−6.05
$CH_3O^- + H_2PO_4^-$	6.37	−5.97

[a] Reaction energies (ΔE) of the displacement reactions in gas phase ($\epsilon = 1$) and in the enzymatic environment ($\epsilon = 20.7$) (kcal/mol).

for the nucleophilic attack of a thiolate and about the change in the reactivity sequence as a function of the protonation state of the electrophile.

Solvent Model. Dianionic compounds are observed in several enzymatic systems, where they become stabilized by electrostatic and hydrogen-bonding interactions within the active site. From this point of view, it is interesting to evaluate the reactivities in proteins by using an implicit solvent model with an appropriate dielectric constant ϵ representing the enzymatic environment. "Appropriate" means that ϵ should depend on the position of the relevant region in the protein[17] and that the optimal value of ϵ depends on the model used[55] (the more of the surrounding enzymatic environment is included explicitly, the lower the optimal ϵ).

The internal, ligand binding side of the loop that forms the active site of ArsC (Figure 1) is readily solvent accessible as depicted in Figure 4. Various studies point out $\epsilon \cong 20$ as an empirical value that maximizes the agreement between calculated and measured pK_a values of surface groups to represent the enzymatic environment.[17,55,56] We therefore choose $\epsilon = 20.7$ (the dielectric constant of acetone, a frequently used organic solvent) in an implicit continuum model (see Theory and Computational Details).

Although we find a declining E versus N curve for the multiply charged anionic compounds in solvent (Figure 2), also the global softness is calculated with eq 13 to be consistent with the methodology followed in gas phase (Tables 2 and 8).

TABLE 8: Softness of the Nucleophiles in the Enzymatic Environment (ϵ = 20.7)[a]

	S	s^-
CH_3S^-	5.458	5.001
CH_3O^-	5.008	4.028

[a] Global softness (S) of CH_3S^- and CH_3O^- (calculated from eq 13) and local softness (s^-) (calculated from eq 9) of the nucleophilic atoms sulfur (S) and oxygen (O) (in a.u.). ($\epsilon = 20.7$.)

TABLE 9: Reactivity in the Enzymatic Environment (ϵ = 20.7) as Measured by Difference in Local Softness[a]

Δs (a.u.)	H_3AsO_4	$H_2AsO_4^-$	$HAsO_4^{2-}$	AsO_4^{3-}
CH_3O^-	2.173	1.513	0.326	1.051
CH_3S^-	3.146	2.486	1.299	0.078
	H_3PO_4	$H_2PO_4^-$	HPO_4^{2-}	PO_4^{3-}
CH_3O^-	2.281	1.757	0.499	1.582
CH_3S^-	3.253	2.829	1.471	0.609

[a] Differences in local softness between As and O, As and S, P and O, and P and S calculated from eq 12. ($\epsilon = 20.7$.)

Applying the HSAB principle (eq 12) gives the following "reactivity matrix" (Table 9)

neutral: As-O > P-O > As-S > P-S

monoanion: As−O > P−O > As−S > P−S

dianion: As−O > P−O > As−S > P−S

trianion: As−S > P−S > As−O > P−O

As far as we have experimental data (relative reactivity of phosphate esters toward an alkoxide and thiolate, relative softness of phosphorus compared to arsenic and of oxygen to sulfur) to compare with our calculated global and local softness and reactivity sequences, our data are in agreement with experiment, with exception of the trianionic species where one expects arsenic to be softer than phosphorus and where our calculations show the opposite (Tables 2, 8, and 9).

The sequence of reaction preference obtained by the calculation of the reaction energies (Table 7) for the mono- and dianionic compounds is equal to the sequence obtained by applying the HSAB principle. This indicates that for the stabilized (dianionic) species in solvent the HSAB principle offers a successful tool to predict reactivities.

The change in reactivity sequence as a function of the protonation state can be noticed in the equal reactivity sequences for the arsenate and phosphate esters and the inversion of the reactivity toward sulfur/oxygen for the trianionic compounds. As in the gas-phase, it can be seen that when the charge of the compound increases, both the global softness of the system and the local softness of the central atom of the compound increase. This parallelism is not necessarily found (Table 2). If the softness of the central electrophilic atoms As and P increases, the reactivity toward the soft sulfur atom increases too, as in the gas-phase (Table 9). The evolution seen in gas-phase of the highest reactivity from the hard−hard combination to the soft−soft combination from, respectively, the neutral to the trianionic form is not observed here. The same applies to the combination with the lowest reactivity.

The reactivity of a thiolate toward a dianionic substrate is higher than that toward a monoanionic substrate. This means that the conclusions drawn from the gas-phase study about the protonation state of the bounded substrates in ArsC and LMWPTPase remain valid in the higher dielectric environment (Table 9) representing the enzymatic active site. This gives a strong argument for the nucleophilic attack of a thiolate on a dianionic rather than on a monoanionic substrate during the first reaction step in the catalysis of ArsC and LMWPTPase.

4. Conclusion

In the gas-phase, where one is confronted with a rising E versus N curve for the multiply charged anionic compounds, we can adopt the HOMO−LUMO gap (eq 13) as a good alternative for the traditional finite difference approach (eq 11) in the calculation of the global softness, even in a DFT context.

Gas-phase model systems representing only the isolated (multiply anionic) compounds involved in the studied enzymatic reactions may provide useful information about the considered enzymatic reaction mechanism. However, results obtained from gas-phase studies on multiply charged anions as is the case in the present study need to be treated with the necessary caution: only when the compounds are stable in gas-phase, which is the case for the neutral and diprotonated species, the reactivity sequences obtained by using the DFT reactivity descriptors in a HSAB context give results in agreement with the computed reaction energy.

Applying the HSAB principle on the stabilized compounds in a solvent model yields a predicted reactivity sequence in agreement with experimental data and calculated reaction energies.

By composing the reactivity matrix of the neutral and deprotonated forms of arsenate and phosphate by theoretical studies in gas-phase as well as in solvent, it is seen that the change in the reactivity sequence is a function of the protonation state of the electrophile. As arsenate and phosphate become more deprotonated (softer), the reactivity predicted by application of the HSAB principle toward the soft sulfur atom increases. This applies to the gas-phase as well as to a solvent model with a dielectric constant that mimics the enzymatic environment. From this observation, we can argue that during the first reaction step catalyzed by ArsC and LMWPTPase the nucleophilic attack of a thiolate occurs on a dianionic rather than on a monoanionic substrate. In the LMWPTPase, the proposal for a dianionic substrate is in line with other studies, indicating the strength of the HSAB principle as a tool in a context of multidisciplinary studies of enzymatic reaction mechanisms.

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Supporting Information Available: Coordinates of the optimized structures (B3LYP/6-31+G**) of the following electrophiles and nucleophiles in gas phase as well as in solvent ($\epsilon = 20.7$): $H_3AsO_4/H_2AsO_4^-/HAsO_4^{2-}/AsO_4^{3-}/H_3PO_4/H_2PO_4^-/HPO_4^{2-}/PO_4^{3-}$ and CH_3O^-/ CH_3S^-. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Zegers, I.; Martins, J. C.; Willem, R.; Wyns, L.; Messens, J. *Nat. Struct. Biol.* **2001**, *8*, 843.

(2) Zhang, Z−Y. *Crit. Rev. Biochem. Mol. Biol.* **1998**, *33*, 1.

(3) (a) Messens, J.; Martins, J. C.; Brosen, E.; Van Belle, K.; Jacobs, D. M; Willem, R.; Wyns, L. *J. Biol. Inorg. Chem.* **2002**, *7*, 146. (b) Messens, J.; Martins, J. C.; Van Belle, K.; Brosens, E.; Desmyter, A.; De Gieter, M.; Wieruszeski, J.-M.; Willem, R.; Zegers, I. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, *531*.

(4) Gladysheva, T.; Liu, J.; Rosen, B. P. *J. Biol. Chem.* **1996**, *271*, 3256.

(5) Hengge, A. C. *Biochem.* **1997**, *36*, 7928.

(6) Czyryca, P. G.; Hengge, A. C. *Biochim. Biophys. Acta* **2001**, *1547*, 245.

(7) Kolmodin, K., Åqvist, J. *FEBS Lett.* **2001**, *498*, 208.

(8) Alhambra, C.; Gao, J. *J. Comput. Chem.* **2000**, *21*, 1192.

(9) Hansson, T.; Nordlund, P.; Åqvist, J. *J. Mol. Biol.* **1997**, *265*, 118.

(10) Kolmodin, K.; Nordlund, P.; Åqvist, J. *Proteins: Struct., Funct., Genet.* **1999**, *36*, 370.

(11) Kolmodin, K.; Åqvist, J. *Int. J. Quantum Chem.* **1999**, *73*, 147.

(12) Asthagiri, D.; Dillet, V.; Liu, T.; Noodleman, L.; Van Etten, R. L.; Bashford, D. *J. Am. Chem. Soc.* **2002**, *124*, 10225.

(13) Lagunas, R.; Pestana, D.; diez-Masa, J. C. *Biochemistry* **1984**, *23*, 955.

(14) Dreuw, A.; Cederbaum, L. S. *Chem. Rev.* **2002**, *102*, 181.

(15) Boldyrev, A. I.; Simons, J. *J. Phys. Chem.* **1994**, *98*, 2298.

(16) Simons, J.; Skurski, P.; Barrios, R. *J. Am. Chem. Soc.* **2000**, *122*, 11893.

(17) Fitch, C. A.; Karp, D. A.; Lee, K. K.; Stites, W. E.; Lattman, E. E.; Garcia-Moreno, E B. *Biophys. J.* **2002**, *82*, 3289.

(18) Pearson, R. G. *Chemical Hardness*; Wiley-VCH: Weinheim, Germany, 1997.

(19) Chattaraj, P. K. *J. Phys. Chem.* **2001**, *105*, 511.

(20) Damoun, S.; van de Woude, G.; Mendez, F.; Geerlings, P. *J. Phys. Chem.* **1997**, *101*, 886.

(21) Ponti, A. *J. Phys. Chem. A* **2000**, *104*, 8843.

(22) Geerlings, P.; De Proft, F. *Int. J. Quantum Chem.* **2000**, *80*, 227.

(23) Parr, R. G.; Yang, W. *Density-Functional Theory of Atoms and Molecules*; Oxford University Press: New York, 1989.

(24) Koch, C. W.; Holthausen, M. C. *A Chemist's Guide to Density Functional Theory*, Second Edition; Wiley-VHC, Weinheim, Germany, 2001.

(25) Parr, R. G.; Yang, W. *Annu. Rev. Phys. Chem.* **1995**, *46*, 701.

(26) Geerlings, P.; De Proft, F.; Langenaeker, W. *Adv. Quantum Chem.* **1999**, *33*, 303.

(27) Chermette, H. *J. Comput. Chem.* **1999**, *20*, 129.

(28) De Proft, F.; Geerlings, P. *Chem. Rev.* **2001**, *101*, 1451.

(29) Geerlings, P.; De Proft, F. *Int. J. Mol. Sci.* **2002**, *3*, 276.

(30) Geerlings, P.; De Proft, F.; Langenaeker, W. *Chem. Rev.* **2003**, *103*, 1793.

(31) Pearson, R. G.; Parr, R. G. *J. Am. Chem. Soc.* **1983**, *105*, 7512.

(32) Chattaraj, P. K.; Lee, H.; Parr, R. G. *J. Am. Chem. Soc.* **1991**, *113*, 1855.

(33) Gázquez, J. L.; Mendez, F. J. *J. Am. Chem. Soc.* **1994**, *98*, 4591.

(34) Yang, W.; Parr, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 6723.

(35) Parr, R. G.; Yang, W. *J. Am. Chem. Soc.* **1984**, *106*, 4049.

(36) Reed, A. E.; Curtiss, L. A.; Weinhold, F. *Chem. Rev.* **1988**, *88*, 899.

(37) (a) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 2. (b) Becke, A. D. J. *Chem. Phys.* **1993**, *98*, 5648.

(38) For a detailed account of this type of basis sets see, for example, Hehre, W.; Radom, L.; Schleyer, P. v. R.; Pople, J. A. *Ab Initio Molecular Orbital Theory*; Wiley: New York, 1986.

(39) De Proft, F.; Martin, J. M. L.; Geerlings, P. *Chem. Phys. Lett.* **1996**, *250*, 393.

(40) (a) Roy, R. K.; Pal, S.; Hirao, K. *J. Chem. Phys.* **1999**, *110*, 8236. (b) Roy, R. K.; Pal, S.; Hirao, K. *J. Chem. Phys.* **2000**, *113*, 1372. (c) Roy, R. K.; Hirao, K.; Krishamurty, S.; Pal, S. *J. Chem. Phys.* **2001**, *115*, 2901. (d) Ayers, P. W.; Morrison, R. C.; Roy, R. K. *J. Chem. Phys.* **2002**, *116*, 8731.

(41) Gázquez, J. L. *J. Phys. Chem. A* **1997**, *101*, 4657.

(42) (a) Nguyen, L. T.; Le, T. N.; De Proft, F.; Chandra, A. K.; Langenaeker, W.; Nguyen, M. T.; Geerlings, P. *J. Am. Chem. Soc.* **1999**, *121*, 5992. (b) Geerlings, P.; De Proft, F. *Int. J. Quantum Chem.* **2000**, *80*, 227. (c) Nguyen, M. T.; Geerlings, P. *J. Org. Chem.* **2001**, *66*, 4316. (d) Nguyen, L. T.; De Proft, F.; Nguyen, M. T.; Geerlings, P. *J. Chem. Soc., Perkin Trans. 2* **2001**, 898. (e) De Proft, F.; Geerlings, P. In *Recent Advances in Density Functional Methods III*; Barone, V., Bencini, A., Fantucci, P., Eds.; World Scientific Publishing Co.: New Jersey, 2002; p 137. (f) De Proft, F.; Vivas-Reyes, R.; Biesemans, M.; Willem, R.; Martin, J. M. L.; Geerlings, P. *Eur. J. Inorg. Chem.*, in press.

(43) Klopman, G. In *Chemical Reactivity and Reaction Paths*; Klopman, G., Ed.; Wiley: New York, 1974; Chapter 1.

(44) Tomasi, J.; Perisco, M. *Chem. Rev.* **1994**, *94*, 2027.

(45) (a) Wiberg, K. B.; Keith, T. A.; Frisch, M. J.; Murcko, M. *J. Phys. Chem.* **1995**, *99*, 9072. (b) Foresman, J. B.; Keith, T. A.; Wiberg, K. B.; Snoonian, J.; Frisch, M. J. *J. Phys. Chem.* **1996**, *100*, 16096. (c) Foresman, J. B.; Frisch, A. E. *Exploring Chemistry with Electronic Structure Methods*, 2nd ed.; Gaussian, Inc: Pittsburgh, PA, 1996. (d) Safi, B.; Choho, K.; De Proft, F.; Geerlings, P. *J. Phys. Chem.* **1998**, *102*, 5253.

(46) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*; Gaussian Inc.: Pittsburgh, PA, 1998.

(47) Boldyrev, A.; Simons, J. *J. Phys. Chem.* **1994**, *98*, 2298.

(48) Koopmans, T. A. *Physica* **1933**, *1*, 104.

(49) Janak, J. F. *Phys. Rev. B* **1978**, *18*, 7165.

(50) Pearson, R. G. *Chemical Hardness*; Wiley-VCH: Weinheim, 1997; Chapter 6.

(51) (a) Politzer, P. *J. Chem. Phys.* **1987**, *86*, 1072. (b) Vela, A.; Gazquez, L. *J. Am. Chem. Soc.* **1990**, *112*, 1490.

(52) (a) Nagle, J. K. *J. Am. Chem. Soc.* **1990**, *112*, 4741. (b) Ghanty, T. K.; Ghosh, S. K. *J. Phys. Chem.* **1993**, *97*, 4951. (c) Fuentealba, P.; Reyes, O. *J. Mol. Struct. (THEOCHEM)* **1993**, *282*, 65.

(53) Politzer, P.; Murray, J. S.; Grice, M. E. In *Chemical Hardness, Structures and Bonding Nature 80*; Springer-Verlag: Berlin, 1993; p 101.

(54) Dantzman, C. L.; Kiessling, L. L. *J. Am. Chem. Soc.* **1997**, *118*, 11715.

(55) Schutz, C.; Warshel, A. *Proteins: Struct., Funct., Genet.* **2001**, *44*, 400.

(56) Dillet, V.; Van Etten, R.; Bashford, D. *J. Phys. Chem. B* **2000**, *104*, 11321.