Table IX. Calculated Electron Populations

| | Z conformer | | | E conformer | | |
|---|---|---|---|---|---|---|
| | $\epsilon = 1$ | $\epsilon = 35.9$ | Δ | $\epsilon = 1$ | $\epsilon = 35.9$ | Δ |
| | | | a. Methyl Formate | | | |
| C1 | 3.977 | 3.970 | −0.007 | 3.963 | 3.968 | +0.005 |
| O2 | 9.391 | 9.401 | 0.010 | 9.368 | 9.397 | +0.029 |
| O3 | 9.352 | 9.352 | 0.000 | 9.340 | 9.346 | +0.006 |
| H4 | 0.975 | 0.978 | +0.003 | 1.007 | 0.988 | −0.019 |
| C5 | 5.253 | 5.260 | +0.007 | 5.240 | 5.283 | +0.043 |
| H6 | 1.021 | 1.004 | −0.007 | 1.002 | 0.986 | −0.016 |
| H7 | 1.016 | 1.019 | +0.003 | 1.041 | 1.018 | −0.023 |
| H8 | 1.016 | 1.019 | +0.003 | 1.041 | 1.018 | −0.023 |
| sum | 32.001 | 32.003 | | 32.002 | 32.004 | |
| | | | b. Methyl Acetate | | | |
| C1 | 4.101 | 4.101 | 0.000 | 4.091 | 4.102 | +0.011 |
| O2 | 9.406 | 9.415 | +0.009 | 9.386 | 9.410 | +0.024 |
| O3 | 9.355 | 9.353 | −0.002 | 9.343 | 9.348 | +0.005 |
| C4 | 5.769 | 5.768 | −0.001 | 5.791 | 5.789 | −0.002 |
| C5 | 5.242 | 5.243 | +0.001 | 5.219 | 5.252 | +0.033 |
| H6 | 1.017 | 1.026 | +0.009 | 0.999 | 1.009 | +0.010 |
| H7 | 1.022 | 1.015 | −0.007 | 1.035 | 1.017 | −0.018 |
| H8 | 1.022 | 1.015 | −0.007 | 1.035 | 1.017 | −0.018 |
| H9 | 1.028 | 1.016 | −0.012 | 1.008 | 1.002 | −0.006 |
| H10 | 1.020 | 1.024 | +0.004 | 1.047 | 1.027 | −0.020 |
| H11 | 1.020 | 1.024 | +0.004 | 1.047 | 1.027 | −0.020 |
| sum | 40.000 | 39.999 | | 39.999 | 39.999 | |

reasonable agreement between calculated and observed gas-phase to solution spectral shifts. Better data would be required in order to have a more detailed test of the calculations, and we plan to obtain such data.

## 7. Conclusions

Polar solvents affect the energy of the $E$ forms of esters considerably more than that of the $Z$ rotamers, leading to smaller energy differences. With methyl formate, the change in $E/Z$ relative energy on going from the gas phase to acetonitrile solution was in very good agreement with that calculated by making use of the surface charge.[16] In the case of methyl acetate, the change in relative energy derived via the reaction field model was in very good agreement with the results of Jorgensen and Houk derived via Monte Carlo simulations.[14] The effect of solvent on the position of the infrared bands of methyl formate and methyl acetate was calculated and was found to be in reasonable agreement with the experimental observations, especially for the C=O stretching mode. These results, along with the others that we have obtained,[12] suggest that the simple reaction field model used in this work will be generally useful, especially for relatively compact molecules that may reasonably be considered as being approximately spherical. Current studies are designed to test the generality of the model.

The effect of temperature on the $E/Z$ equilibrium constant for methyl formate was calculated and was found to be quite small because of the change in dielectric constant of acetonitrile with temperature. It seems likely that this will have a major effect on the $\Delta H^\circ$ and $\Delta H^\ddagger$ values derived from temperature coefficients of equilibrium constants or rate constants for processes in which there is a significant change in dipole moment.

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# Electronegativities in Situ, Bond Hardnesses, and Charge-Transfer Components of Bond Energies from the Topological Theory of Atoms in Molecules

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**Abstract:** Rigorous definitions for electronegativities of atoms and functional groups in molecules, bond hardnesses, and the charge-transfer components of the bond energies are proposed. The definitions rely upon values of total energies and their derivatives calculated for molecules composed of fragments with a controlled degree of charge transfer. Such calculations, in which the atomic or fragment charges are obtained with the help of the topological theory of atoms in molecules, are easily accomplished by adding appropriate Lagrange multiplier terms to the electronic Hamiltonian. Numerical examples that are given for 23 different systems indicate that the bond hardnesses are mostly transferable, but because of the electric field generated by the molecular environment the electronegativity differences are not.

## Introduction

The concept of electronegativity[1] is central to understanding and explaining a multitude of chemical phenomena. As with any other quantity, in order to realize and harness its full predictive and explanatory potential, it is essential to replace the vague statement of electronegativity as "the power of an atom in a molecule to attract electrons to itself"[1a] by a rigorous definition. Over the last 50 years numerous quantitative definitions of electronegativity have appeared in the chemical literature. Although as early as in 1961 Iczkowski and Margrave related electronegativity to the linear coefficient in the *fitted* polynomial expansion for the total energy in terms of the total charge,[1b] it was not until 1978, when the connection between electronegativity, $\chi$, and the first partial derivative of the total energy, $E$, with respect to the number of electrons, $N$ (at a constant external potential, v),

$$\chi = -\left(\frac{\partial E}{\partial N}\right)_v \tag{1}$$

was made, that the full understanding of the concept of electronegativity was achieved.

As the derivative (1) is discontinuous at integer values of $N$, in practice the above definition is replaced by a finite-difference approximation, yielding

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$$\chi = (1/2)(I + A) \qquad (2)$$

where $I$ and $A$ are respectively the ionization potential and electron affinity of the system in question. The "absolute" electronegativity[2] obtained from eq 2 is identical with that arising from Mulliken's definition.[3] Similarly, by applying the finite-difference approach to the expression

$$\eta = (1/2)\left(\frac{\partial^2 E}{\partial N^2}\right)_v \qquad (3)$$

one arrives at

$$\eta = (1/2)(I - A) \qquad (4)$$

as the definition of the absolute hardness.[4]

Expressions 2 and 4 allow one to calculate the values of electronegativity and hardness for any isolated atom, ion, or molecule. The connection between electronegativity and the concepts of the density functional theory (DFT) identifies $\chi$ as the negative chemical potential. Therefore, upon formation of a molecule from two fragments, electron transfer from the fragment of a lower electronegativity to that of a higher electronegativity is expected to continue until the electronegativities of both fragments become equal.[5,6] This electronegativity equalization principle holds the promise of making electron distributions in molecules and the related properties, such as bond ionicities, amenable to both prediction and understanding.

Unfortunately, the fact that electronegativities of atoms in molecules are not the same as those of isolated atoms has not been adequately appreciated by the chemical community. In particular, despite the well-established (thanks to many early works, see ref 7 for a recent review) fact that electronegativity depends on both the atomic charge and molecular environment, it is not uncommon to come across explanations of chemical phenomena based on electronegativities of isolated atoms, leaving a misleading impression (very prevalent among chemistry students) that electronegativity is some kind of a fundamental constant for an atom. Another common myth is that the variability of atomic electronegativities can be fully accounted for by considering the valence-state values of $\chi$.[8]

In our continuous effort to rigorously quantify common chemical concepts, we have recently introduced bias-free definitions of covalent bond orders,[9] molecular similarities,[10,11] and contact interatomic separations that replace the empirical van der Waals radii in the assessment of steric crowding in molecules.[12] In this paper, we propose a rigorous and consistent approach to defining atoms and molecular fragments in situ, electronegativities of atoms and groups in molecules, and bond hardnesses. In addition, we introduce a well-defined measure of the charge-transfer component of the bond energy.

## Formation of a Molecule from Its Constituting Fragments

Let A and B be two disjoint molecular fragments (or atoms). Let AB be a molecule in its ground-state equilibrium geometry. It is assumed that the positions of the nuclei of A and B exactly match those of AB. Formation of the molecule AB can be conceptually divided into two steps. In the first step, the electron distributions of A and B are adjusted from those corresponding to the isolated fragments (or atoms) to those reflecting their mutual interactions, but no electron transfer between A and B is allowed. In the second step, electron transfer does occur, resulting in the equilibrium electron distribution. In considering the second step, it is also possible to go one step further by assuming various degrees of electron transfer and investigating the accompanying changes in the total energy of AB.

For the sake of simplicity, let us consider A and B that bear no electric charges when not interacting with each other. A generalization to isolated A and B being ions is straightforward and has no impact upon the final conclusions. The quantity

$$Q_{CT} = Q_A = -Q_B \qquad (5)$$

measures the amount of charge transfer. The total energy $E_{AB}(Q_A,Q_B) \equiv E_{AB}(Q_{CT})$ in the vicinity of $Q_{CT} = 0$ can be formally expanded in a double Taylor series as

$$E_{AB}(Q_A,Q_B) = E_{AB}(0,0) + \chi_A Q_A + \chi_B Q_B + \eta_{AA} Q_A^2 + \eta_{BB} Q_B^2 + 2\eta_{AB} Q_A Q_B + \ldots \qquad (6)$$

or

$$E_{AB}(Q_{CT}) = E_{AB}(0,0) + (\chi_A - \chi_B)Q_{CT} + (\eta_{AA} + \eta_{BB} - 2\eta_{AB})Q_{CT}^2 + \ldots \qquad (7)$$

In eqs 6 and 7, $\chi_A$ and $\chi_B$ can be immediately identified as the electronegativities of A and B in the molar environment of AB. Similarly, one finds $\eta_{AA}$ and $\eta_{BB}$ to be the corresponding hardnesses of A and B. One should note the similarity between eq 6 and the expansion of Iczkowski and Margrave[1b] for isolated atoms, the difference being the presence of a cross-term involving $\eta_{AB}$.

Expansions 6 and 7 have been widely used in construction of many semiempirical treatments aimed at prediction of atomic charges and bond ionicities. Several approximations to eqs 6 and 7 have been proposed. First of all, although the higher-order terms in expansion 6 for isolated atoms have been recently discussed,[13] many researchers have truncated eqs 6 and 7 (or their generalizations to more than two atoms) at the quadratic terms. Such an approach produces simple formulae for the atomic charges that are the foundation of the electronegativity equalization method (EEM).[14] Second, despite the fact that reliable estimates of the off-diagonal element of the hardness matrix, $\eta_{AB}$, indicate that it is usually quite large,[15] many researchers have neglected it entirely. The $\chi$ and $\eta$ constants in eqs 6 and 7 have often been equated with the electronegativities and hardnesses pertaining to isolated fragments. Improving the estimates of $\chi$ by correcting the electronegativities for the electrostatic potential (usually simulated by that generated by point charges) due to the counterfragment has been advocated.[16] Finally, some researchers have argued for partial electronegativity equalization, in which the charges resulting from minimization of the total energy with respect to $Q_{CT}$ are multiplied by some (to large degree arbitrary) constants.[17]

All of the above approaches are useful in approximate predictions of atomic charges and bond ionicities. However, when used in conjunction with analyses of accurate electronic wave functions, they can be a potential source of considerable confusion. This is so, because they are based on different partitionings of the energy and therefore the quantities entering eqs 6 and 7 are expected to depend strongly on the approximation used. From the above discussion it is clear that rigorous definitions of electronegativities and hardnesses of atoms and fragments in molecules are possible only if (1) the assumption of a strictly quadratic dependence of the total energy on the fragment (or atomic) charges is abandoned, (2) the charges of fragments (or atoms) in molecules

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are rigorously defined, and (3) there is a possibility of calculating the total energy of the molecule in question as a well-defined function of the relevant fragment (or atomic) charges. In the following section of this paper we address all three aforementioned subjects.

## Molecular Fragments in Situ

When the amount of charge transfer assumed in the second step of the process of molecular formation (see above) does not correspond to the equilibrium charge distribution, the respective wave function of the molecule AB, $\Psi$, does not describe a real quantum-mechanical system. Nevertheless, it is still possible to calculate the total energy $E_{AB}(Q_{CT})$ for any reasonable value of $Q_{CT}$ by carrying out a constrained search[18,19]

$$E_{AB}(Q_{CT}) = \min_{\Psi \to Q_{CT}} \langle \Psi | \hat{H}_{AB} | \Psi \rangle \tag{8}$$

where $\hat{H}_{AB}$ is the Hamiltonian describing AB and the wave function $\Psi$ is assumed to be normalized and correspond to a proper number of electrons. When $\Psi$ is constrained to yield a given value of $Q_{CT}$ (different from $Q^0_{CT}$, the $Q_{CT}$ pertaining to the equilibrium electron distribution), it describes a fictitious molecule AB. In particular, when $Q_{CT}$ is set to zero, $\Psi$ describes the molecule in question with A and B being fragments in situ, i.e. with their nuclei in proper positions, but no electron transfer allowed. Since $E_{AB}(Q_{CT})$ can now be computed for any $Q_{CT}$ in the vicinity of zero, the difference between the electronegativities of A and B in situ is readily afforded by (compare eq 7)

$$\left.\frac{\partial E_{AB}(Q_{CT})}{\partial Q_{CT}}\right|_{Q_{CT}=0} = \chi_A - \chi_B = \Delta\chi_{AB} \tag{9}$$

Similarly, the second derivative gives

$$\left.\frac{\partial^2 E_{AB}(Q_{CT})}{\partial Q^2_{CT}}\right|_{Q_{CT}=0} = 2(\eta_{AA} + \eta_{BB} - 2\eta_{AB}) = \kappa_{AB}(0) \tag{10}$$

the bond hardness. One may also calculate the analogous quantity at $Q_{CT} = Q^0_{CT}$, obtaining $\kappa_{AB}(Q^0_{CT})$. The difference between $\kappa_{AB}(0)$ and $\kappa_{AB}(Q^0_{CT})$ measures the functional deviation of $E_{AB}(Q_{CT})$ from the simple quadratic expression 7.

Another important quantity related to $E_{AB}(Q_{CT})$ is the charge-transfer component of the bond energy, $E^{CT}_{AB}$, which we define as

$$E^{CT}_{AB} = E_{AB}(0) - E_{AB}(Q^0_{CT}) \tag{11}$$

$E^{CT}_{AB}$ is a measure of the ionic contribution to the bond energy. Definition 11 is of a more general character than that given by Morokuma,[20] as the latter one is applicable only to systems with weak interactions, such as molecular complexes. One should note that, by virtue of the variational principle, $E^{CT}_{AB} \geq 0$.

What remains is a proper definition for the charges of molecular fragments. For the fragments of the real molecule, the definition of Bader[21] is a logical choice due to its rigorous character. For fragments that do not correspond to the electron distribution of the real molecule (such as the fragments in situ), the constrained wave function does not describe a stationary state of a free quantum-mechanical system at equilibrium. Therefore, quantum-mechanical relations such as the virial and Ehrenfest theorems are not expected to be satisfied for the entire molecule or any of its fragments unless $Q_{CT} = Q^0_{CT}$. For this reason, we propose that $Q_{CT}$ be calculated according to Bader's topological theory of atoms in molecules,[21] but *with the fragment basins* $\Omega_A$ *and* $\Omega_B$ *identical with those of the real molecule AB instead of those corresponding to the constrained* $\Psi$. Such a definition greatly simplifies the actual calculations without impinging upon the physical rigor of Bader's theory.

## Computational Implementation

At the Hartree–Fock (HF) level, the determination of the constrained wave function and the corresponding total energy is easily accomplished by adding a Lagrange multiplier term to the Fock matrix of the unperturbed ($Q_{CT} = Q^0_{CT}$) system

$$F_{pq}(Q_{CT}) = F_{pq}(Q^0_{CT}) + \lambda\langle p|q\rangle_A \tag{12}$$

where $\langle p|q\rangle_A$ is a sum of the elements of the atomic overlap matrices[22] pertaining to the fragment A of the unperturbed molecule in question. The degree of charge transfer is obtained from

$$Q_{CT} = Z_A - \sum_{pq} P_{pq}\langle p|q\rangle_A \tag{13}$$

where $Z_A$ is the total nuclear charge of A and $P_{pq}$ is an element of the HF one-electron density matrix. The total HF energy, $E_{AB}(Q_{CT})$, is calculated in the usual way. A similar approach can be used in conjunction with electron correlation methods such as MP2, CCSD, or MCSCF.

No matter which method is used to calculate $E_{AB}(Q_{CT})$, the difference of electronegativities in situ, $\Delta\chi_{AB}$ (eq 9), is conveniently obtained as the value of the Lagrange multiplier $\lambda$ at which $Q_{CT} = 0$. The bond hardness, $\kappa_{AB}(0)$ (eq 10), is readily afforded from

$$\kappa_{AB}(0) = \left.\frac{\partial\lambda(Q_{CT})}{\partial Q_{CT}}\right|_{Q_{CT}=0} \tag{14}$$

The value of $\kappa_{AB}(Q^0_{CT})$ is similarly obtained as the first derivative at $Q_{CT} = Q^0_{CT}$.

In order to compute the above quantities within the Hartree–Fock approximation, links 401 and 601 of the GAUSSIAN88 suite of codes[23] were appropriately modified. The actual calculations proceed as follows: First, the SCF wave function of the unperturbed molecule and the corresponding energy are computed. The atomic overlap matrices are then evaluated by numerical integrations within the respective atomic basins.[22,24] These AOMs are read and combined into overlap matrices pertaining to the fragments A and B by the modified link 401. The charge-constrained SCF calculations commence with sweeping $\lambda$ around its zero value, thus yielding $\kappa_{AB}(Q^0_{CT})$ by means of a symmetric finite-difference approximation for the relevant first derivative. The knowledge of both $Q^0_{CT}$ and $\kappa_{AB}(Q^0_{CT})$ makes it possible to guestimate the value of $\lambda$ that corresponds to $Q_{CT} = 0$. Starting from this guess, the charge-constrained calculations are repeated until $Q_{CT}$ is equal to zero within a reasonable accuracy (such as $10^{-5}$) and then concluded with a final sweep of $\lambda$ that yields $\kappa_{AB}(0)$. In practice, the entire process usually requires between 7 and 9 single-point evaluations of the SCF energy.

## Numerical Examples

In order to illustrate the aforedescribed theoretical approach and discuss trends among the computed electronegativities, hardnesses, and charge-transfer components of the bond energies, we carried out a series of calculations at the HF/6-311G** level of theory. Thirteen molecules with geometries optimized at the same level of theory were considered, giving rise to 16 cases of C—H bonds and 5 cases of C—C bonds, together with one C=C bond and one C≡C bond (Table I). Among the combinations with A = H, the CN group was found to be the most electronegative with the corresponding $\Delta\chi_{AB} = -7.13$ eV. The $C_2H_5$ group is the most electropositive with $\Delta\chi_{AB} = 1.67$ eV. For the five cases involving the methyl group as fragment A, the cyano group is again the most electronegative with $\Delta\chi_{AB} = 9.86$ eV. In general, on the basis of the data listed in Table I, one may conclude

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**Table I.** The HF/6-311G** Group Charges, Electronegativities, Bond Hardnesses, and Charge–Transfer Components of the Bond Energy

| A | B | $Q_{CT}$ | $\chi_A - \chi_B$ (eV) | $E_{AB}^{CT}$ (kcal/mol) | $\kappa_{AB}(Q_{CT}^0)$ (eV) | $\kappa_{AB}(0)$ (eV) |
|---|---|---|---|---|---|---|
| H | CN | 0.203 | -7.13 | 17.0 | 37.46 | 33.59 |
| H | CCH | 0.144 | -4.97 | 8.4 | 35.99 | 33.59 |
| H | $CCCH_3$ | 0.130 | -4.47 | 6.8 | 35.50 | 33.39 |
| H | $CF_3$ | 0.078 | -2.67 | 2.4 | 34.93 | 33.59 |
| H | $CH_2CN$ | 0.036 | -1.12 | 0.5 | 31.78 | 31.28 |
| H | $CHF_2$ | 0.024 | -0.81 | 0.2 | 33.40 | 32.98 |
| H | $CH_2CF_3$ | 0.010 | -0.33 | 0.0 | 32.17 | 32.01 |
| $H_{(op)}$ | $CH_2CHO$ | 0.007 | -0.23 | 0.0 | 30.97 | 30.75 |
| H | $CH_2CCH$ | 0.006 | -0.17 | 0.0 | 31.08 | 30.92 |
| H | $CHCH_2$ | -0.011 | 0.34 | 0.0 | 25.23 | 32.01 |
| H | $CH_2F$ | -0.012 | 0.38 | 0.1 | 32.20 | 32.39 |
| H | CHO | -0.013 | 0.41 | 0.1 | 30.79 | 30.92 |
| H | $COCH_3$ | -0.025 | 0.77 | 0.2 | 30.17 | 30.57 |
| $H_{(ip)}$ | $CH_2CHO$ | -0.031 | 0.97 | 0.3 | 31.51 | 31.82 |
| H | $CH_3$ | -0.035 | 1.09 | 0.4 | 31.29 | 31.64 |
| H | $CH_2CH_3$ | -0.054 | 1.67 | 1.0 | 30.86 | 31.46 |
| $CH_3$ | CN | 0.343 | -9.86 | 39.6 | 29.71 | 26.94 |
| $CH_3$ | CCH | 0.244 | -6.67 | 19.0 | 27.99 | 26.16 |
| $CH_3$ | $CF_3$ | 0.230 | -6.69 | 17.8 | 29.58 | 28.95 |
| $CH_3$ | CHO | 0.073 | -1.96 | 1.7 | 26.77 | 26.94 |
| $CH_3$ | $CH_3$ | 0.000 | 0.00 | 0.0 | 26.76 | 26.76 |
| $CH_2$ | $CH_2$ | 0.000 | 0.00 | 0.0 | 12.81 | 12.81 |
| CH | $CCH_3$ | 0.391 | -4.83 | 21.8 | 12.45 | 12.31 |

that the C—C bond is softer than the C—H bond. The C≡C bond is softer than the C=C bond, which in turn is substantially softer than the C—C bond. This means that electron transfer between two molecular fragments connected through a bond linking two carbon atoms is greatly facilitated by the presence of $\pi$ electrons in the bond vicinity.

An example of the dependence of $E_{AB}(Q_{CT})$ on $Q_{CT}$ is displayed in Figure 1 for the H–CN bond formation. Although the dependence is approximately quadratic, the curve is noticeably unsymmetrical with respect to $Q_{CT} = Q_{CT}^0$.

## Discussion and Conclusions

The fact that the proposed definition is incapable of providing absolute electronegativities is not a serious handicap, as the magnitudes of charge transfer in molecules depend only on the electronegativity differences. From inspection of Table I, one immediately recognizes that the electronegativity differences are not fully transferable between different molecules. Therefore, although in both the A = H and $CH_3$ (Me) series the electronegativity decreases in the direction CN > $CF_3$ > CHO > $CH_3$, the differences between $\Delta\chi_{HB}$ and $\Delta\chi_{MeB}$ are not constant and they do not equal $\Delta\chi_{HMe}$. Moreover, the CCH fragment is substantially more electronegative in the HCCH molecule than $CF_3$ is in $HCF_3$, whereas the electronegativities of the CCH and $CF_3$ fragments in the $CH_3CCH$ and $CH_3CF_3$ molecules are comparable, the latter being slightly higher.

The above observations are easily rationalized by the fact that, although the A–B bond is the only (at least as far as the topological theory of atoms in molecules[21] is concerned) direct link between the fragments A and B, the residual (through space) interactions are strong enough to mutually influence the electronegtivities of fragments A and B. The operational mechanism is, of course, the electrostatic field generated by each fragment that distorts the electron distribution of its counterpart. This phenomenon also readily explains the positional dependence of electronegativities, such as the difference between the $\Delta\chi_{AB}$ for the in-plane and out-of-plane hydrogens (denoted in Table I by $H_{(ip)}$ and $H_{(op)}$, respectively) in the H–$CH_2CHO$ system.

As one may conclude from the limited data presented in the previous section of this paper, the dependence of the bond hardnesses extends beyond that on the atoms linked directly by the bond in question. For the series of 16 C–H bonds, $\kappa_{AB}(0)$ varies within ca. 10%. The corresponding figure for the 5 C–C bonds studied is also ca. 10%. This means that the assumption of the $\kappa_{AB}(0)$ values being constant for a given bond, although probably sufficiently reliable for crude semiempirical theories,[14] is not adequate for accurate predictions of fragment (or atomic) charges. The same is true for approximating the $E_{AB}(Q_{CT})$ dependence on $Q_{CT}$ by a quadratic function. For values of $Q_{CT}^0$ as small as 0.3, the difference between $\kappa_{AB}(0)$ and $\kappa_{AB}(Q_{CT}^0)$ can be as large as 10% (see the $CH_3CN$ entry in Table I).

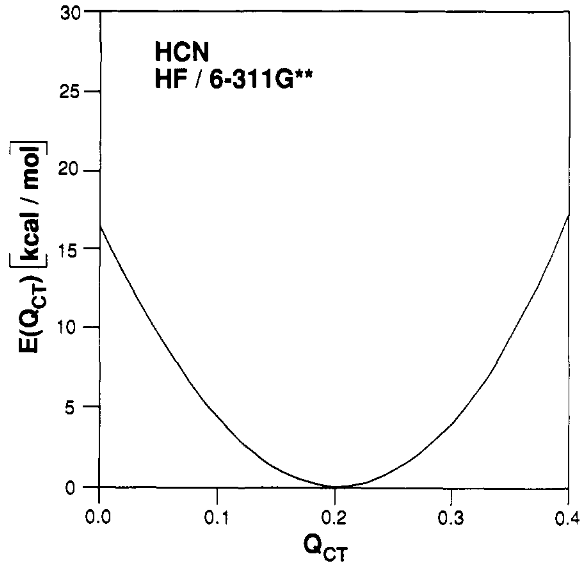

**Figure 1.** The dependence of $E_{AB}(Q_{CT})$ on $Q_{CT}$ for the HCN molecule (A = H, B = CN). Note that the energy scale has been shifted to yield $E_{AB}(Q_{CT}^0) = 0$.

Since the range of charge transfers encountered in the systems under study is not very large, the bond energies are found to be dominated by covalent interactions. This does not mean that the calculated charge-transfer components of the bond energies are negligible. For the $CH_3$–CN bond, the value of $E_{AB}^{CT}$ is 39.6 kcal/mol, or ca. 30–40% of the total bond dissociation energy.

The final comment should be devoted to the dependence of the computed quantities on the level of theory used. As inclusion of electron correlation tends to reduce bond ionicities,[25] one may expect the magnitudes of $\Delta\chi_{AB}$ and $E_{AB}^{CT}$ to be somewhat overestimated by the HF approximation. Although the HF level of theory is appropriate for the charge-constrained calculations on bonds that are not too ionic, for strongly ionic bonds, such as for example that in the LiF molecule, forcing neutrality of the fragments requires a multiconfigurational (MCSCF) description. This is so, because the neutral fragments in situ give rise to a biradicaloid character of the resulting systems. In a sense, forcing the charge-transfer component to vanish in highly ionic molecules is not unlike dissociating the respective bonds. Studies on such systems will be the subject of a separate study.

Although not fully transferable, the computed electronegativity differences undoubtedly reflect the electronic structures of molecules better than the conventional electronegativities, as the latter either pertain to average or individual valence states of free atoms and functional groups or are derived with the help of various semiempirical schemes. Our formulation of $\Delta\chi_{AB}$, $\kappa_{AB}$, and $E_{AB}^{CT}$ is based on a single underlying definition that does not even assume that the energy dependence on the degree of charge transfer is exactly quadratic. In fact, the deviations from a quadratic dependence can be easily assessed within our approach. Our definition provides an alternative to the previously proposed semiempirical treatments.[14,26] Finally, unlike the electronegativity scale proposed recently by Boyd et al.,[27] which also pertains to

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fragments in situ, our approach does not use any indirect semiempirical relationships to evaluate $\chi$, relying directly on the fundamental relationship, eq 1, instead.

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# Mechanism-Based Inactivation of *Escherichia coli* β-Hydroxydecanoyl Thiol Ester Dehydrase: Assignment of the Imidazole $^{15}$N NMR Resonances and Determination of the Structure of the Alkylated Histidine

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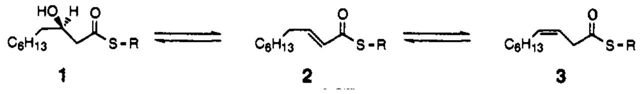
**Abstract:** Nitrogen-15 nuclear magnetic resonance spectroscopy was used to determine the structure of the active-site histidine-70 adduct formed when β-hydroxydecanoyl thiol ester dehydrase from *Escherichia coli* reacts with the mechanism-based inactivator *S*-(3-decynoyl)-*N*-acetylcysteamine (3-decynoyl-NAC). In order to obtain the amount of labeled enzyme necessary for spectral studies, the *fabA* gene, which encodes dehydrase, was overexpressed to give dehydrase as 15–20% of soluble protein. To simplify the interpretation of the NMR spectra, the non-active-site histidine residue His-129 was converted to an asparagine residue using site-directed mutagenesis. The specific activity and response of the mutant to 3-decynoyl-NAC are unaltered. It is known that type β ("pyridine-like") nitrogens in imidazoles resonate 60–80 ppm downfield of type α ("pyrrole-like") nitrogens. To assign the imidazole nitrogen resonances in dehydrase, wild-type and mutant enzymes were labeled with $^{15}NH_4Cl$ or with [$^{15}N^{\delta1}$]histidine. Analysis of the $^{15}$N NMR spectra allowed the assignment of the resonances of the imidazole nitrogens of His-129 and His-70. For His-70, the spectra show that $N^{\delta1}$ resonates upfield of $N^{\epsilon2}$ in the native enzyme and is therefore a type α nitrogen. In the inactivated enzyme the signals are reversed, and $N^{\epsilon2}$ is a type α nitrogen. These results demonstrate that $N^{\epsilon2}$ of His-70 becomes alkylated upon inactivation of dehydrase with 3-decynoyl-NAC and thus is the probable locus of active-site basicity in the normal reactions catalyzed by dehydrase. In addition, the imidazole nitrogen chemical shifts suggest that $N^{\delta1}$ may be involved in a hydrogen bond in native dehydrase but that $N^{\epsilon2}$ is not. The mechanistic implications of this are discussed.

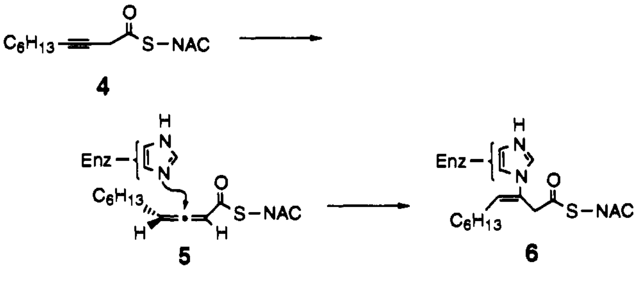
## Introduction

β-Hydroxydecanoyl thiol ester dehydrase catalyzes reactions at a critical branch point in the biosynthetic pathway for unsaturated fatty acids in *Escherichia coli* and (presumably) other strictly or facultatively anaerobic bacteria.[1] Dehydrase catalyzes the interconversion of 3-hydroxydecanoyl, (*E*)-2-decenoyl, and (*Z*)-3-decenoyl thiol esters (**1**, **2**, and **3**, respectively; Scheme I). In vivo, (*E*)-2-decenoyl-ACP[2] is reduced and then elongated to saturated fatty acids, while direct elongation of (*Z*)-3-decenoyl-ACP leads to the common monounsaturated fatty acids. Extensive mechanistic studies have shown that the allylic rearrangement[3] and dehydration reactions catalyzed by dehydrase are suprafacial[4] and syn[5] processes, respectively. Thus, a single active-site acid/base group is implied.[6,7]

**Scheme I**



**Scheme II**



Chemical modification studies as well as examination of the pH–rate profile of dehydrase (inflection points at pH 7 and 10)[8] provide compelling evidence for the involvement of a histidine residue in the catalytic mechanism as well as equivocal evidence

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[2] Abbreviations used: ACP, acyl carrier protein; dNTP, deoxynucleoside triphosphate; IPTG, isopropyl β-D-thiogalactopyranoside; MOPS, 4-morpholinopropanesulfonic acid; NAC, *N*-acetylcysteamine; PCR, polymerase chain reaction; ssDNA, single-stranded DNA; TPPI, time proportional phase incrementation.

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