Quantum Chemical Design of Hydroxyurea Derivatives For the Treatment of Sickle Cell Anemia

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I. Introduction

Sickle cell anemia is an inherited disorder in which red blood cells become stiff and sickle-shaped. This condition is caused by defective hemoglobin that clusters together, forming long, rod-like structures.[1] The abnormal red blood cells cannot freely move through small blood vessels and thus cause blockages that deprive organs and tissues of oxygen.[2,3] A study published in 2003 established that the use of hydroxyurea therapy decreases mortality among sickle cell patients by forty percent and significantly reduces pain and acute chest crises.[2] Hydroxyurea produces an increase of fetal hemoglobin, which prevents the polymerization of sickle hemoglobin.[1,4] It is also a source of nitric oxide (NO), a messenger molecule needed to maintain normal blood flow and pressure.[4-8] Hydroxyurea reacts with hemoglobin by first forming a nitroxide radical.[6] It then undergoes a series of reactions to produce the nitric oxide needed to increase fetal hemoglobin.[4,6,7] Although the production of NO can proceed through various pathways, the process always requires the removal of the hydrogen atom from the OH group of hydroxyurea.

This study concerns the formation of the nitroxide radical, which is the rate-limiting reaction in the process by which hydroxyurea treats sickle cell disease.[5] Huang, Kim-Shapiro, and King have recently shown by experiment that derivatives of the

hydroxyurea molecule can form NO-producing radicals more quickly than hydroxyurea can produce nitroxide.[7] In this work, the ability of different hydroxyurea derivates to generate NO-producing radicals is explored computationally. The molecular energies of hydroxyurea derivatives and their corresponding radicals were computed using a variety of quantum chemical methods, including Hartree-Fock theory, density functional theory, and correlated wavefunction (or *ab initio*) methods, such as many-body perturbation theories and coupled-cluster methods. Specifically, energy difference between hydroxyurea and the nitroxide radical was compared with the energy difference between each hydroxyurea derivative and its radical. As explained in this report with thermodynamic and kinetic arguments, the smaller energy differences favor the formation of the radical. A table "ranks" the derivatives according to their energy differences and compares the results with experimentally determined rate constants when available. The results of the study show that all nineteen hydroxyurea derivatives form NO-producing radicals faster than hydroxyurea. One of these derivatives, Zileuton, approved by the Food and Drug Administration for the treatment of asthma, has been shown to increase fetal hemoglobin in sickle cell patients.[9]

II. Background

Hydroxyurea produces nitric oxide (NO) through a three-electron oxidation.[6] This requires a single-electron oxidation of hydroxyurea to first produce the nitroxide radical, which may disproportionate to form NO (Figure 1). The radical may also undergo another single-electron oxidation, forming C-nitrosoformamide before decomposing to NO.[6]

Figure 1

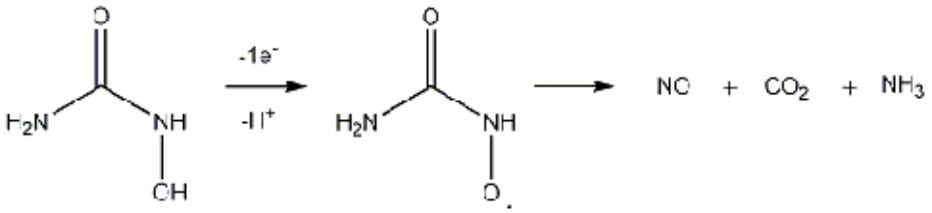


Figure 1: Hydroxyurea produces the nitroxide radical, which then disproportionates to form NO.

Hydroxyurea forms the nitroxide radical by interacting with hemoglobin.[1] First, it oxidizes oxyhemoglobin (Fe^{+2}-O_2) to yield methemoglobin (Fe^{+3}) and the nitroxide radical. Hydroxyurea then reacts with methemoglobin, forming a low-spin methemoglobin-hydroxyurea complex.[5,6] This complex produces deoxyhemoglobin and another nitroxide radical that decomposes to nitric oxide as previously described (Figure 2). Hydroxyurea can increase fetal hemoglobin levels by using the nitric oxide produced through a guanylate cyclase pathway as well as through many other pathways.[4,7] The reactions described occur only at moderate rates and require a large excess of hydroxyurea,[7] suggesting that other molecules similar to hydroxyurea may be more reactive.

Figure 2

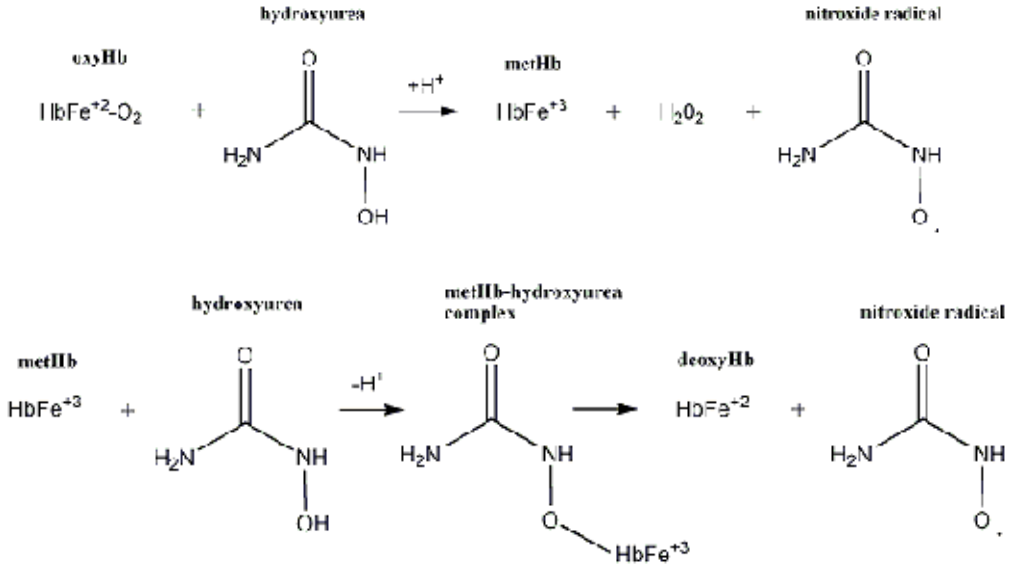


Figure 2: Hydroxyurea forms the nitroxide radical by oxidizing oxyhemoglobin to methemoglobin. It also reacts with the methemoglobin to form a methemoglobin complex, which then produces deoxyhemoglobin and another nitroxide radical.

III. Methodology

In the human body, the conversion of hydroxyurea to the nitroxide radical is assisted by the reaction of hemoglobin, either from oxyhemoglobin to methemoglobin or from methemoglobin to deoxyhemoglobin. Alone, the removal of the hydrogen atom from hydroxyurea to form the nitroxide radical requires such a considerable amount of energy that virtually no nitroxide would form, but because the transformation of hemoglobin is thermodynamically favorable, the probability of hydroxyurea to produce the nitroxide radical increases within the body. Additionally, the removal of nitroxide by its decomposition to nitric oxide assists nitroxide production because, by LeChatlier's principle, the reaction compensates for the loss of product by creating more product.

However, a large excess of hydroxyurea is necessary for the reaction to occur because the equilibrium constant still significantly favors hydroxyurea.

Because the removal of a hydrogen atom from a hydroxyurea derivative does not cause an appreciable change in pressure or volume of the system, the change in enthalpy is equal to the energy difference. Furthermore, since the entropy for the reaction also remains essentially constant, the free energy change is equivalent to the change in electronic energy (Figure 3). The electronic energy differences between the hydroxyurea derivatives and their radicals *alone* indicate the favorability of their equilibrium constants for radical formation because the ratio of the equilibrium constant for the conversion of hydroxyurea to nitroxide to the equilibrium constant for the conversion of a hydroxyurea derivative to its radical is independent of any hemoglobin reactions. Hemoglobin has the same thermodynamic role in both reactions, and thus, its concentration cancels in the ratio of the equilibrium constants for the two reactions, as shown in Figure 4. This means that comparing electronic energy differences between derivatives and their radicals is an accurate method for ordering the molecules based on their thermodynamic formation of radicals.

Figure 3

Consider the relations $\Delta H = \Delta E + \Delta(PV)$ and $\Delta G = \Delta H - T\,\Delta S$.

If $\Delta P = 0$, $\Delta V = 0$, $\Delta S = 0$, then

$$\Delta H \approx \Delta E$$

and

$$\Delta G \approx \Delta E.$$

Figure 3: If there are no changes in pressure, volume, or entropy, the free energy change may be equated to the change in energy between hydroxyurea or any of its derivatives and their corresponding radical.

Figure 4

$$K_1 = [\text{nitroxide}] / [\text{hydroxyurea}]$$

$$K_2 = [\text{methemoglobin}]\,[\text{peroxide}] / [\text{oxyhemoglobin}]$$

$$K_x = K_1\,K_2$$

$$K_3 = [\text{radical}] / [\text{hydroxyurea derivative}]$$

$$K_y = K_3\,K_2$$

$$K_x / K_y = (K_1\,K_2) / (K_3\,K_2)$$

$$= (\,K_1 / K_3\,)$$

Figure 4: Let K_x represent the equilibrium constant for the conversion of hydroxyurea to nitroxide, and let K_y represent the equilibrium constant for the conversion of a hydroxyurea derivative to its radical. Dividing K_x by K_y will cause the equilibrium constant for the hemoglobin reactions cancel out. Therefore, the reactions of hydroxyurea and its derivatives may be compared with one another without considering the hemoglobin reactions.

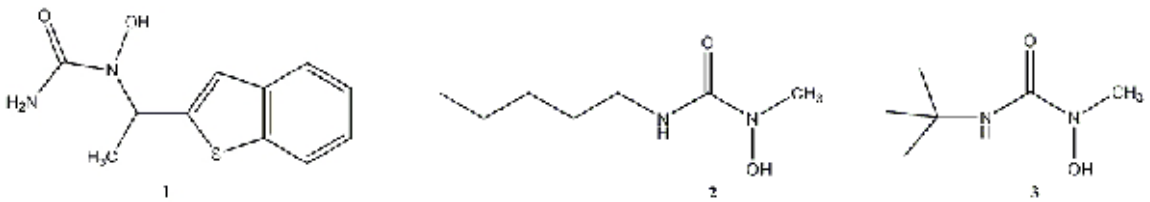
Without hemoglobin, the potential energy surface of the process of removing hydrogen from hydroxyurea increases monotonically with the distance of the hydrogen atom from the remaining hydroxyurea molecule. In the presence of hemoglobin, therefore, the removal of hydrogen would be expected to have an energy barrier corresponding to the hydroxyurea-hemoglobin transition state. The reaction rates of the different hydroxyurea derivatives will consequently depend on the height of the transition-state barrier. To compare these rates, it can be assumed that changes in the energy difference between hydroxyurea derivatives and their radicals translate to similar changes in the barrier height. Although equilibrium constants may be compared without consideration of the interactions between the hydroxyurea derivative and hemoglobin, reaction rates may be influenced by such interactions between the hydroxyurea derivative

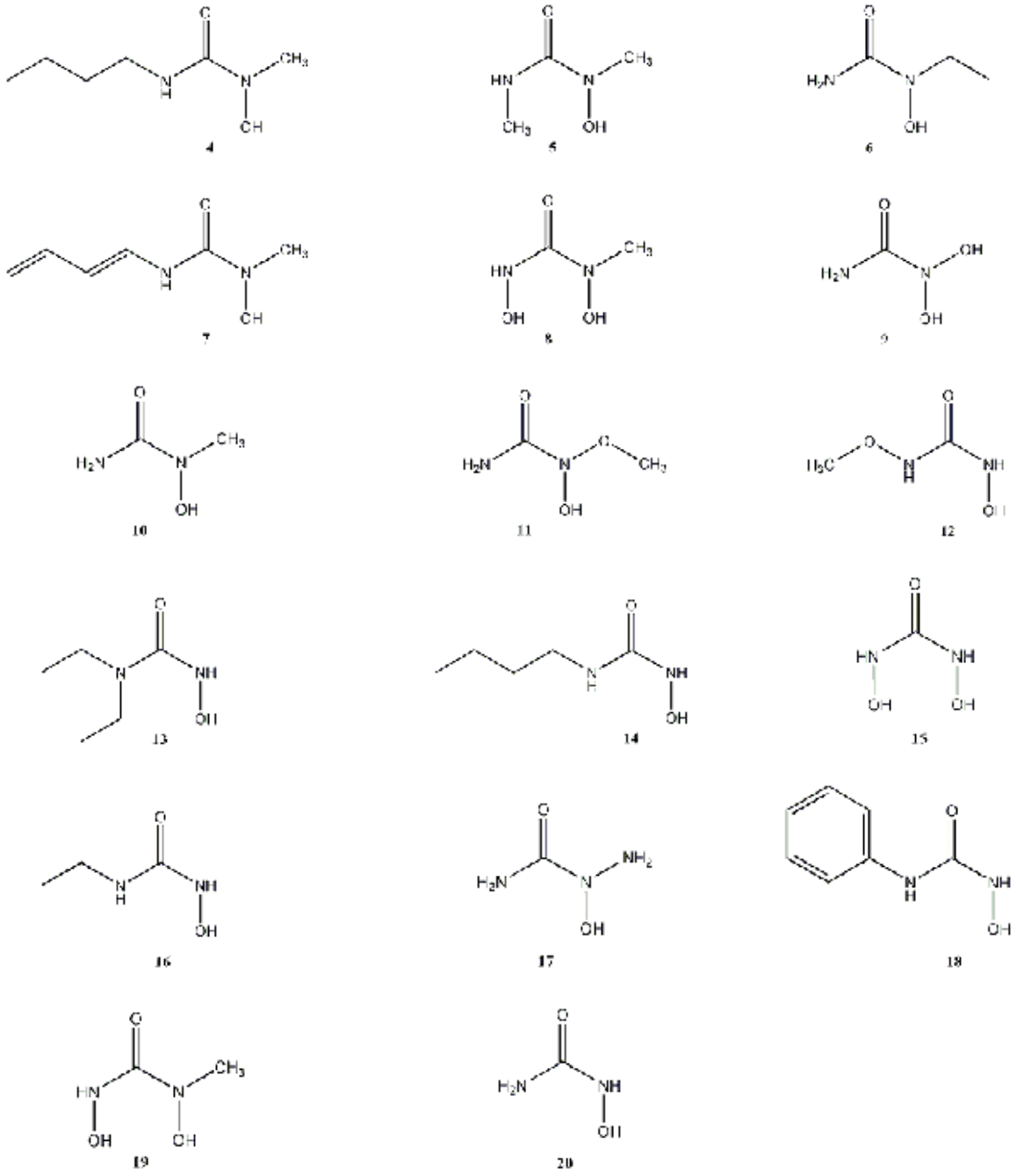
and hemoglobin, such as steric effects or kinetic enhancements. Comparing only hydroxyurea derivatives and their radicals allows us to determine an estimate of the relative reaction rates from well-defined electron structure calculations without the treatment of hemoglobin in the transition state.

Various methods were employed to calculate the electronic energies of the hydroxyurea derivatives and their radicals. In addition to Hartree-Fock, correlated wavefunction methods were used, including second- and fourth-order perturbation theories (MP2 and MP4), coupled-cluster singles-doubles (CCSD), and coupled cluster singles-doubles with a perturbative triples correction (CCSD(T)). Three different correlation-exchange functions (PBEPBE, G96PBE, and B3LYP) were used within density functional theory. For each method, calculations were done in three Pople basis sets (6-31G, 6-31G*, and 6-31G**) using the quantum chemistry code Gaussian.[10] Equilibrium geometries were found for all molecules at the Hartree-Fock level of theory.

Table I

Hydroxyurea (20) and nineteen of its derivatives were tested in this study, including Zileuton (1), an asthma medication that has been shown to increase fetal hemoglobin in sickle cell patients. Derivatives 8 and 19 have the same structure, but the radical of derivative 8 forms on the CH_3 side, while the radical of derivative 19 forms on the opposite side.





IV. Calculations and Results

Hydroxyurea and nineteen derivatives were tested in this study (Table I), including some of those proposed and chemically tested by S. B. King.[5] Every derivative

was investigated using the previously described methods. Table II provides a comparison of the energy difference between hydroxyurea and nitroxide with the energy difference between hydroxyurea derivative 10 and its radical. The results of using three correlated wavefunction methods are given as well as the calculations using three density functional methods. Specifically, second-order many-body perturbation theory (MP2), coupled cluster singles doubles (CCSD), and coupled cluster singles doubles with a pertubative triples correction (CCSD(T)) are the correlated methods shown, and PBEPBE, G96PBE, and B3LYP are the density functional methods shown. The Hartree-Fock (HF) method, which does not include correlation energy, is also displayed. This method predicts that the modification of hydroxyurea will lower the energy difference between the molecule and the radical by 29.5 kJ/mol, whereas the correlated wavefunction methods predict a larger decrease in the energy difference. The MP2, CCSD, and CCSD(T) methods yield an energy reduction by 20.6 kJ/mol, 64.4 kJ/mol, and 106.8 kJ/mol respectively. The density function methods, PBEPBE, G96PBE, and B3LYP, show a decrease in the energy differences by 51.7 kJ/mol, 51.4 kJ/mol, and 48.8 kJ/mol respectively. The energy methods with electron correlation included predict a much larger difference between the molecules and their radicals. Correlation energy accounts for more than fifty percent of the energy difference between molecules and radicals for some of the hydroxyurea derivatives. The density function theory calculations tend to indicate an even larger energy difference than the correlated wavefunction methods. The table shows these differences among calculations performed in a 6-31G** basis set. Calculations done in the 6-31G and 6-31G* basis sets produced similar trends.

Table III provides a comparison of the energy differences between the molecule and radical found using the B3LYP density functional method in basis sets 6-31G, 6-31G*, and 6-31G**. All the molecules, arranged in order of increasing energy difference, have smaller energy differences than hydroxyurea (molecule 20). Derivatives with a group substituted for one or both of the hydrogen atoms on the side opposite the hydroxide group showed the smallest improvement over hydroxyurea in the energy difference between molecule and their derivative (12-16, 18). Of these molecules, the methyl ether derivative (12) produced the least energy difference, while the derivative with a butyl group (14) and well as one with an ethyl group (16) also produced some of the smaller energy differences. The least improvement was observed from the substitution of a phenyl group (18).

Substitutions on the hydroxide side of the molecule tended to produce smaller and thus more favorable energy differences. The addition of an ethyl group, a hydroxide group (9), methyl group (10), or a methyl ether group (11) greatly improved the energy difference. Within this class, an amine substitution (17) produced the least improvement in energy difference, while the hydroxyurea derivative Zileuton (1) yielded the most significant improvement over hydroxyurea in the study. Although Zileuton's energy difference of 1608.8 kJ/mol might not seem much more favorable than the 1670.0 kJ/mol energy difference of hydroxyurea, it is important because small changes in energy have large and significant effects on equilibrium and kinetics.

The derivatives with substitutions on both nitrogen atoms (2-5, 7, 8) generally produce even smaller energy differences between molecule and radical. Each has a methyl group attached to the hydroxide side. The substitution of a phenyl group (2)

produces the most favorable results, although the addition of a butyl (4) or methyl group (5) also yields a very small molecule–radical energy difference. A branched alkane substitution (3) slightly improved the difference from the butyl group (4), while an unsaturated chain (7) yielded a greater energy difference. Although longer alkane chains generally produce small molecule-radical energy differences, the steric effect from additional chains may be unfavorable for the transition state.

The experimental rate constants available for five molecules tested in this study shown in Table III (10, 13, 14, 18, 20) generally agree with these calculations. The rate constant for the formation of nitroxide from hydroxyurea (7.54×10^{-4} min^{-1}) is slower than any of the derivatives, which agrees with the results of this study. Derivative 10 generates its radical about two hundred times faster than hydroxyurea. This experimental result highlights how small energy differences, in this case a 48.8 kJ/mol difference in molecule-radical energy gaps between hydroxyurea and derivative 10, have significant effects on reaction rates. The experimental rate constants also agree with the calculations for derivatives 10, 13, and 14, although derivative 18 has a much more favorable rate constant than the one expected from its energy calculations. Its rate is similar to derivative 10 while its computed energy difference is larger by approximately 24.2 kJ/mol. This discrepancy may have arisen because the phenyl group may have kinetically enhanced the transition state through a stabilization. This effect cannot be predicted without examination of the methemoglobin-hydroxyurea complex.

Table II

The molecule-radical energy difference of hydroxyurea is greater than that of Derivative 10, suggesting that Derivative 10 may form a radical more quickly than hydroxyurea.

	Energy Differences (kJ/mol) between Hydroxyurea and Radical in Basis Set 6-31G**			Energy Differences (kJ/mol) between Derivative 10 and Its Radical in Basis Set 6-31G**		
Method	Energy of Hydroxyurea	Energy of Radical	Difference	Energy of 10	Energy of Radical 10	Difference
HF	-784457.4	-782884.7	1572.7	-886960.2	-885417.0	1543.2
MP2	-786626.3	-785025.6	1600.6	-889601.3	-887980.0	1621.3
CCSD	-786688.3	-784988.8	1699.5	-889577.0	-887942.0	1635.1
CCSD(T)	-786756.0	-785019.8	1736.2	-889605.6	-887976.2	1629.3
PBEPBE	-787923.8	-786271.7	1652.1	-891004.7	-889404.4	1600.4
G96PBE	-788433.6	-786774.1	1659.5	-891590.5	-889982.4	1608.1
B3LYP	-788771.5	-787101.4	1670.0	-892006.8	-890385.5	1621.2

Table III

Hydroxyurea derivatives are ranked in order of increasing molecule-radical energy differences in three basis sets. Any known experimental rate constants are also provided.

Energy Differences (kJ/mol) between Hydroxyurea Derivatives and their Radicals				
	6-31g	6-31g*	6-31g**	
Molecule	Energy Difference	Energy Difference	Energy Difference	Rate Constant (min^{-1})
1	1592.6	1594.9	1608.8	
2	1596.7	1600.9	1614.8	
3	1597.4	1601.3	1615.4	
4	1597.6	1601.6	1615.7	
5	1598.5	1602.8	1616.8	
6	1599.2	1602.8	1616.9	
7	1600.0	1604.3	1618.2	
8	1603.8	1605.3	1619.5	
9	1608.5	1606.8	1620.3	
10	1602.8	1607.8	1621.2	$3.93 \times 10^{-2} \pm 1.68 \times 10^{-3}$
11	1607.3	1607.2	1621.3	
12	1603.7	1608.6	1622.5	
13	1603.3	1612.3	1625.8	$2.26 \times 10^{-3} \pm 8.16 \times 10^{-5}$
14	1604.9	1612.2	1626.0	$8.56 \times 10^{-3} \pm 1.87 \times 10^{-4}$
15	1610.4	1615.0	1629.0	
16	1606.1	1615.6	1629.2	
17	1620.7	1621.1	1634.5	
18	1621.8	1631.9	1645.5	$6.24 \times 10^{-2} \pm 6.18 \times 10^{-3}$
19	1615.2	1632.3	1646.7	
20	1639.7	1656.3	1670.0	$7.54 \times 10^{-4} \pm 2.16 \times 10^{-5}$

V. Conclusions

This study has investigated the effects of modifying hydroxyurea to increase the generation of nitric oxide-producing radicals for the treatment of sickle cell anemia. Electronic energies were calculated for derivatives of hydroxyurea and their radicals, and the energy differences between the molecules and the radicals have been used to compare their thermodynamic and kinetic favorability for radical formation by hydrogen abstraction. Every derivative tested had a smaller and thus more favorable energy difference than hydroxyurea. Alone, this energy difference cannot exactly predict the reaction rate of radical formation because the transition state involves both hydroxyurea and hemoglobin. The hemoglobin-hydroxyurea interactions may produce kinetic enhancements. However, to a first-order of approximation, the energy differences between each molecule and its radical offer an estimate of the molecule-radical conversion rate. Experimental rate constants confirm that some derivatives of hydroxyurea form radicals more quickly than hydroxyurea. A computational investigation has significant advantages because many molecules can be tested without a time-consuming and possibly expensive laboratory synthesis.

The results of this study indicate that correlation energy significantly affects the magnitude of electronic energy calculations. The energy differences between each molecule and its radical are significantly larger with electron correlation. It can be concluded, therefore, that correlation energy is crucial to accurately compare energy differences between molecules. Both the basis set size and the correlation method affects the calculated energy differences, but these changes are small compared to those from the inclusion of correlation energy.

The results of this study suggest that hydroxyurea derivatives may form radicals at a faster rate than hydroxyurea. In particular, the asthma medication Zileuton seems to have the fastest reaction rate of any derivative tested. A recent study also indicates that this drug increases fetal hemoglobin in sickle cell patients. The derivatives of hydroxyurea may also improve treatment of sickle cell anemia by producing fewer undesirable side effects. To fully determine the affects of substituting a hydroxyurea derivative for hydroxyurea to treat sickle cell disease, the activity of the molecule during the reactions following radical formation must also be investigated. Computation of hydroxyurea derivatives and their radicals by quantum chemistry provides a unique approach to considering modifications of hydroxyurea for improved treatment of sickle-cell anemia.

References

(1) M. W. Rupon, S. R. Domingo, S. V. Smith, *et al*, Biophysical Chemistry, 84, 1-8, 2000.

(2) L. De Franceschi and P. G. B. Rossi, Haematologica, **89**, 348-353 (2004).

(3) S. K. Ballas and E. D. Smith, Blood, **79**, 2154-2158 (1992).

(4) V. P. Cokic, R. D. Smith, B. B. Beleslin-Cokic, *et al*, J. Clin. Invest. **111**, 231-235 (2003).

(5) J. Huang, Z. Zou, D. B. Kim-Shapiro, S. K. Ballas, and S. B. King, J. Med. Chem. **46**, 3748 (2003).

(6) S. B. King, Current Medical Chemistry, **10**, 437 (2003).

(7) J. Huang, D. B. Kim-Shapiro, and S. B. King, J. Med. Chem. **47**, 3496 (2004).

(8) C. Halsey and I. A. G. Roberts, Br. J. Haematology, 120, 177-182, 2003.

(9) J. Haynes Jr, B. S. Baliga, B. Obiako, S. Ofori-Acquah, and B. Pace, Blood, **103**, 3945 (2004).

(10) M. J. Frisch, G. W. Trucks, H.B. Schlegel *et al*., Gaussian 98 (Gaussian, Inc., Pittsburgh, PA, 1998).