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Methemoglobinemia Caused by 8-Aminoquinoline Drugs: DFT Calculations Suggest an Analogy to H_4B's Role in Nitric Oxide Synthase

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Supporting Information

ABSTRACT: We suggest a possible mechanism of how 8-aminoquinolines (8-AQ's) cause hemotoxicity by oxidizing hemoglobin to methemoglobin. In our DFT calculations, we found that 5-hydroxyprimaquine is able to donate an electron to O_2 to facilitate its conversion to H_2O_2. Meanwhile, Fe(II) is oxidized to Fe(III) and methemoglobin is formed. In this mechanism, the 8-AQ drug plays a similar role as that of H_4B in nitric oxide synthase. Furthermore, our study offers an approach to inform the design of less toxic antimalarial drugs.

There is a great need for new antimalarial drugs that are active against the dormant liver stages of Plasmodium parasites.[1,2] At present, the only FDA-approved drug which can kill liver hypnozoites is primaquine[3] (**1**) (Figure 1), a member of the 8-aminoquinoline (8-AQ) family. *In vivo*, primaquine is believed to form a number of metabolites, such as carboxyprimaquine[4] (**2**), 5-hydroxyprimaquine[5] (5-HPQ, **3**), and 6-methoxy-8-aminoquinoline[6] (**4**) (Figure 1). A major concern regarding primaquine and its metabolites is that they can cause life-threatening hemolysis in G6PD-deficient patients.[7] The hemolysis may be related to the propensity of 8-AQ metabolites to oxidize hemoglobin to methemoglobin, an Fe(III) protein which is unable to carry oxygen.[8] The reaction also results in the formation of reactive oxygen species (ROS), such as hydrogen peroxide. Since primaquine was first synthesized in 1946,[9] numerous efforts have been made to reduce the toxicity of 8-AQ drugs.[10] Unfortunately, our knowledge of such toxicity has been limited by a lack of understanding of its chemical mechanism.[4,11]

In this communication, we suggest a possible mechanism for the methemoglobinemia of 8-AQ drugs. Considering the process of converting O_2 to H_2O_2, it is apparent that in addition to two protons, two electrons must be supplied to the π^* orbital of O_2. The question is: where do the two electrons come from? Obviously, iron is able to provide one electron with Fe(II) being oxidized to Fe(III), which results in the conversion of hemoglobin to methemoglobin. The source of the second electron, however, is not clear. In this work we hypothesize that an 8-AQ metabolite may provide this second electron, itself being converted into a radical cation. Interestingly, if this were true, the 8-AQ metabolite then plays a similar role to that of tetrahydrobiopterin (H_4B) in nitric oxide synthase (NOS). NOS is an iron-heme containing enzyme that catalyzes the formation of nitric oxide from L-arginine. It has been found that NOS needs the cofactor H_4B during catalysis to transfer an electron to the $Fe-O_2$ moiety.[12] In particular, Shaik and co-workers[13] studied the active site protonation states of NOS using QM/MM methods and found that when O_2 is doubly protonated to form the $Fe-H_2O_2$ complex, H_4B is converted to a radical cation and transfers an electron to aid the formation of H_2O_2. Similarly, when complexed with hemoglobin, an 8-AQ or an 8-AQ metabolite could assist the formation of H_2O_2 analogously as does H_4B in NOS.

To test this hypothesis, we performed a density functional theory (DFT) study on unprotonated, singly protonated and doubly protonated hemoglobin−8-AQ complexes. 5-HPQ (**3**) was chosen for this study because it is known to cause methemoglobinemia directly and is able to form H_2O_2.[14] By contrast, primaquine itself appears to require metabolic activation to elicit methemoglobin formation.[10] The terminal amine of the 5-HPQ's 8-amino alkyl chain was assumed to be protonated to match physiological pH and the asymmetric carbon alpha to the 8-amine (see Figure 1) was chosen in the *S* form. The initial 5-HPQ−hemoglobin complex was obtained by docking 5-HPQ into hemoglobin using the Glide program.[15] The crystal structure (PDB: 2D5X),[16] in which a ligand 2-[4-(3,5-dichlorophenylureido) phenoxy]-2-methylpropionic acid makes a hydrogen bond with the propionate group of heme, was used for the docking. Detailed docking procedures are provided in the Supporting Information. The docked structure showed an interaction of the terminal 8-amino $-NH_3^+$ group of 5-HPQ with the carboxylic side chain of heme (Figure 2a), most likely due to the formation of a strong electrostatic interaction. On the basis of this result, a chemical model to be used for DFT calculations was derived (Figure 2b). All DFT calculations were performed using the spin unrestricted approach. Geometries were optimized using the Jaguar program[17] at the B3LYP[18] level with the LACVP* basis set. Relative energies were obtained at those geometries using Jaguar's Poisson−Boltzmann self-consistent reaction field method with a dielectric constant (ε) of 4.0 at the B3LYP/LACV3P** level of theory. The above computational approach to study metalloenzymes has recently been applied to similar systems and reviewed in depth.[19]

Figure 3 shows the optimized structures of unprotonated, singly protonated, and doubly protonated O_2−hemoglobin−5-HPQ complexes. Upon optimization, the terminal $-NH_3^+$

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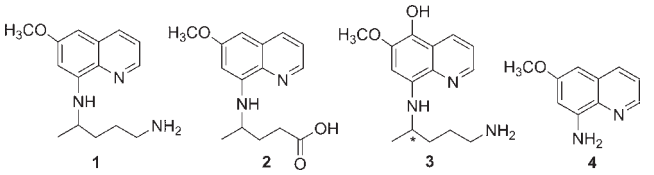

Figure 1. Schematic illustration of primaquine and metabolites.

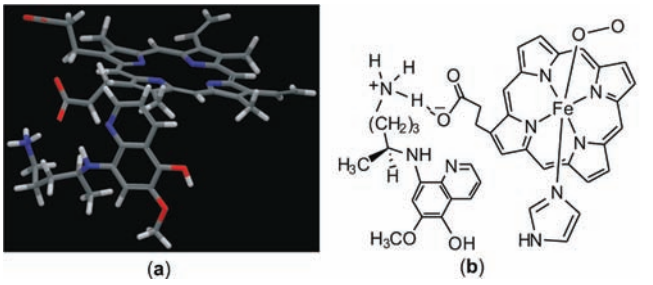

Figure 2. (a) Interaction of 5-HPQ with hemoglobin from docking into 2D5X. For clarity, only 5-HPQ and heme are shown. The side chain terminal amine was found to be in close proximity to the heme carboxyl moiety, whereas the alkyl group at the 8-position and the quinoline ring are more than 3 Å away from the heme and form hydrophobic interactions with Leu86 and Leu83. (b) Chemical model used for DFT calculations.

group of 5-HPQ in all the protonation states transfers a proton to the carboxylic group of heme, forming a $-H_2N\cdots HOOC-$ hydrogen bond. For the unprotonated complex, the lowest energy structure is in the singlet spin state (Table 1). In this spin state, an electron is transferred from iron to one of the π^* orbitals of O_2 to form $Fe^{III}-O_2^{\bullet-}$. The O_2 moiety shows superoxide character (electron configuration $\sigma_x^2\pi_x^2\pi_y^2\pi^*_x{}^2\pi^*_y{}^1\sigma^*_x{}^0$) with the O−O bond length at 1.27 Å. Thus, the O_2 is in the one-electron reduced state. This electronic assignment is in agreement with computational studies on similar systems.[20−24] It should be noted that in all the possible spin states, the spin density on 5-HPQ is 0.00, suggesting that no electron is transferred from 5-HPQ to O_2.

In the singly protonated complex, the lowest energy structure is in the triplet spin state. The singlet spin state, however, lies only marginally higher in energy by 0.3 kJ mol^{-1}. In the NOS system complexed with OOH, the singlet and triplet states were similarly found to be very close in energy.[13] The affinity of the unprotonated complex to accept one proton was calculated to be 1133.5 kJ mol^{-1} using the free energies[25] of the unprotonated and singly protonated complexes in the ground state. In the triplet state, iron is still in the Fe(III) state. The O−O bond is further lengthened to 1.44 Å, indicating that it is essentially a single bond. Thus, an electron has been promoted to the other π^* orbital of $O_2^{\bullet-}$ to convert it to O_2^{2-} with electron configuration $\sigma_x^2\pi_x^2\pi_y^2\pi^*_x{}^2\pi^*_y{}^2\sigma^*_x{}^0$. Hence, the O_2 is now in the two-electron reduced state. From the calculated spin distribution (Table 1), it can be concluded that this electron is partially supplied by each of heme and 5-HPQ, with 5-HPQ contributing ~0.64 electrons. In the triplet, quintet and septet states, the unpaired electron shared between heme and 5-HPQ aligns ferromagnetically with that in iron, while in the single state, they align antiferromagnetically.

In the doubly protonated complex, the lowest energy structure is also in the triplet state. The affinity of the singly protonated complex to accept an additional proton was calculated to be

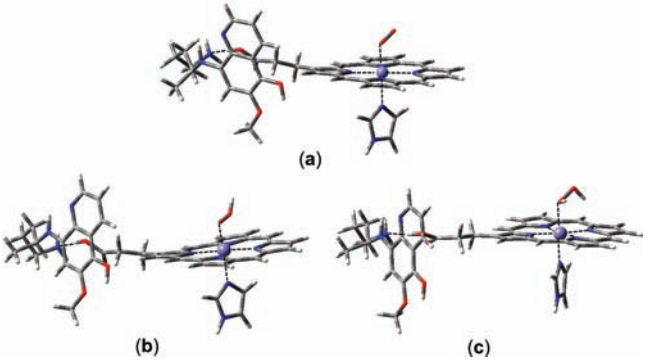

Figure 3. Optimized structures of (a) unprotonated, (b) singly protonated, and (c) doubly protonated O_2 hemoglobin−5-HPQ complexes in the lowest energy spin states.

1126.6 kJ mol^{-1} using the free energies[25] of the singly protonated and doubly protonated complexes in the ground state. The proton affinity of H_2O was calculated to be 997.2 kJ mol^{-1} at the same level. The affinities of O_2 to accept protons are larger than that of H_2O, suggesting that the hemoglobin−5-HPQ bound O_2 is more basic than H_2O and should be able to be protonated by H_3O^+. In the triplet spin state of the doubly protonated complex, iron remains in the Fe(III) state. The O−O bond lengthens slightly further to 1.45 Å and the spin densities on both oxygen atoms are 0.00. Thus, as for the singly protonated complex, O−O is closed-shell and single bonded with electron configuration $\sigma_x^2\pi_x^2\pi_y^2\pi^*_x{}^2\pi^*_y{}^2\sigma^*_x{}^0$. However, in addition to the electron donated by iron, the second electron transferred to the π^* orbital of O_2 is now purely provided by 5-HPQ, which is converted to a radical cation, as shown from its calculated spin density of 1.01.

In a separate set of gas phase calculations, we fixed the lengths of the two terminal N−H bonds to prevent the proton transfer to the carboxylic acid side chain of the heme. This restriction to the $-H_3N^+\cdots{}^-OOC-$ interaction, however, did not affect the conclusions obtained (see Supporting Information). In addition, to take into account that the binding site is located at the protein surface, single point calculations were performed on selected structures with ε of 80.37 (representing water), but in this case also similar results were obtained (Supporting Information).

These results show that 5-HPQ indeed plays a similar role for hemoglobin as H_4B does for NOS. It is, in fact, an even better electron donor than H_4B. In Shaik and co-workers' study[13] on NOS, the process of electron transfer from H_4B was only observed when O_2 is doubly protonated. In contrast, when O_2 is singly protonated in the hemoglobin−5-HPQ complex, 5-HPQ donates nearly two-thirds of an electron to O_2. This may be explained in part by the fact that 5-HPQ has a lower ionization energy than H_4B. The gas phase calculation at the B3LYP/6-311+G(2df,p)//B3LYP/6-31G(d,p) level shows that H_4B has an ionization energy of 593.9 kJ mol^{-1}. However, that of 5-HPQ is 13.2 kJ mol^{-1} lower in energy at 580.7 kJ mol^{-1}. Thus, 5-HPQ is more capable of donating an electron than H_4B.

Furthermore, it should be noted that the binding pocket is located at the surface of hemoglobin. In addition to the $-H_2N\cdots HOOC-$ hydrogen bond, the ligand interacts hydrophobically with the amino acid residues of the protein. The relatively nonspecific hydrophobic interactions suggest that the proposed chemical mechanism may be general to the class of aromatic compounds that can form a hydrogen bond with the

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Table 1. Relative Energies (kJ mol^{-1}) and Spin Densities for the Unprotonated (O_2), Singly Protonated (OOH), and Doubly Protonated (H_2O_2) Species in all the Possible Spin Multiplicities

			spin density				
structure	M	ΔE	Fe	O_{inner}	O_{outer}	heme	5-HPQ
O_2	1	0.0	1.37	−0.56	−0.73	−0.08	0.00
	3	16.3	1.10	0.33	0.62	−0.04	0.00
	5	32.3	2.14	0.94	0.97	−0.08	0.00
	7	17.5	3.80	1.00	0.99	0.17	0.00
OOH	1	0.3	0.84	0.19	0.02	−0.42	−0.63
	3	0.0	0.85	0.19	0.03	0.32	0.64
	5	62.1	3.06	−0.16	−0.07	0.62	0.59
	7	31.4	4.13	0.40	0.09	0.75	0.61
HOOH	1	11.8	1.05	0.00	0.00	−0.03	−1.01
	3	0.0	1.06	0.00	0.00	−0.04	1.01
	5	38.7	3.03	0.02	0.00	−0.12	1.01
	7	10.2	4.26	0.04	0.00	0.59	1.01

carboxylic arm of heme via an amine or a hydroxyl group. Indeed, certain small compounds such as aniline and its metabolites are also able to catalyze the formation of methemoglobin *in vivo*.[26] These processes may follow a similar mechanism to that discussed in this communication.

In the present work, we suggest a possible explanation of the methemoglobinemia caused by 8-AQ drugs. Both iron and 8-AQ donate an electron to the π^* orbital of O_2. This thus facilitates the formation of H_2O_2. In the meantime, Fe(II) is converted to Fe(III). Therefore, methemoglobin, which cannot bind O_2, is formed. Notably, in this mechanism 8-AQ plays a similar role as that of H_4B in NOS. In principle, modification of the exocyclic substituents of 8-AQ's will affect their electron donating ability, which suggests a rational plan for discovery of new, less toxic 8-AQ drugs.

■ ASSOCIATED CONTENT

Supporting Information. The detailed docking procedures and the top 10 binding poses generated by the Glide SP method, the effects of proton transfer and more polarized environment, and optimized *xyz* coordinates of all structures considered in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

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