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Effect of antimalarial drug primaquine and its derivatives on the ionization potential of hemoglobin: A QM/MM study

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Abstract

We used quantum mechanics/molecular mechanics calculations to test if antimalarial primaquine (PQ) and its derivatives aid the conversion of hemoglobin to methemoglobin by binding to hemoglobin and merely lowering hemoglobin's ionization potential (IP). Our results showed that PQ and its derivatives do not significantly lower the hemoglobin IP, disproving the hypothesis.

Primaquine (PQ, Fig. 1) has long been used clinically as an antimalarial drug. However, a serious side effect for this drug is that its metabolites, such as 5-hydroxyprimaquine (5-OH-PQ, Fig. 1), are able to cause hemolysis, particularly in glucose-6-phosphate dehydrogenase deficient patients. Such a process was thought to be caused in part by the conversion of hemoglobin to methemoglobin, which simultaneously generates reactive oxygen species (ROS), such as superoxide radical and hydrogen peroxide.¹⁻³ In order to further develop less hemolytic antimalarial drugs, it is essential to understand the chemical mechanism of methemoglobinemia caused by primaquine metabolites, which is unfortunately not clear despite more than sixty years of tremendous studies⁴⁻¹¹ since PQ was first synthesized in 1946.¹²

Considering that methemoglobinemia and the generation of ROS are redox processes, electron transfer is of particular importance for this type of reaction. Recently, we have studied the methemoglobinemia mechanism from two angles by considering the possibilities that the PQ metabolites act either as electron acceptors or electron donors. Regarding the former possibility, we found that the mechanism that PQ metabolites accept an electron from Fe(II) in hemoglobin to convert it to methemoglobin is not feasible, because we were able to show¹³ that 5-OH-PQ, a metabolite that is known to generate more methemoglobin than the parent compound,^{14, 15} has essentially the same adiabatic electron affinity as PQ. Considering the latter possibility, we found that 5-OH-PQ is indeed able to donate an electron to the hemoglobin-bound O₂, which facilitates its conversion to H₂O₂ and simultaneously generates methemoglobin by oxidizing Fe(II) to Fe(III).^{16,17} Although these two mechanisms have considered the possibility that PQ metabolites are *directly* involved in the redox processes, a third possible mechanism is that the PQ metabolites play an indirect role by simply increasing the ability of Fe(II) in hemoglobin to lose an electron to form

Fe(III), i.e., lowering hemoglobin's ionization potential, upon binding to hemoglobin. In this proposed mechanism, the electron lost by Fe(II) in hemoglobin would be transferred to some other species, perhaps an unknown cofactor or protein. This possibility can be investigated by calculating the energetic difference between hemoglobin and methemoglobin as each in turn is complexed with PQ or its derivatives, which represents the energy needed to ionize Fe(II) in hemoglobin. In this work, such ionization potentials of hemoglobin with several PQ derivatives (possible or known metabolites) bound were calculated by the quantum mechanics/molecular mechanics (QM/MM) method in order to study the feasibility of this third possible mechanism.

All QM/MM calculations were performed using the QSite program.¹⁸ Geometry optimizations were performed at the same level as in our previous study, specifically B3LYP/LACVP/OPLS_2005.¹⁶ Relative energies were obtained by performing single point calculations at the B3LYP/LACV3P+**/OPLS_2005 level based on the optimized geometries. The B3LYP method has been widely used in studying the properties of heme-related proteins and shown to be able to provide reliable geometries and energies.¹⁹⁻²²

The initial PQ...hemoglobin bound complex was obtained from our previous docking calculations,¹⁷ in which the preferred binding pose was found to involve an electrostatic interaction between the terminal -NH_3^+ of PQ and the carboxylic group of heme (cf. Fig. 2). Based on this complex, the oxidation state of iron was set to either Fe(II) or Fe(III), to represent hemoglobin or methemoglobin, respectively. The QM region is shown in Fig. 2 and included the iron, heme, a histidine residue coordinated with iron and modeled as an imidazole, and PQ or one of its derivatives. The total charge of the QM region was zero when the oxidation state of iron was set to be +2. In addition, another complex without PQ bound was considered, since it is able to serve as a basis for comparison. In order to be consistent, the carboxylic group of heme in this complex was set to be protonated so that the QM region was also neutral when the oxidation state of iron was set to be +2.

As noted above, two systems were studied, one with iron in the Fe(II) state and one with iron in the Fe(III) state. We first considered the complexes without PQ present, since this is able to serve as a reference point to study the role of PQ and its metabolites. When iron was set to be in the Fe(II) state, the singlet, triplet and quintet spin states were considered. Similarly to what was found in previous studies on hemoglobin and other heme related systems,²³⁻²⁵ the quintet spin state was found to be the lowest in energy (Table 1). In this spin state, two of the $3d$ electrons of iron are paired while the other four $3d$ electrons are unpaired. After iron loses an electron to become Fe(III), the doublet, quartet and sextet spin states are possible. The quartet spin state, with three unpaired electrons in iron's $3d$ orbital, was found to be the ground state. Using the ground state energies of the Fe(II) and Fe(III) systems, the energy needed to ionize hemoglobin to form methemoglobin was calculated to be $606.4 \text{ kJ mol}^{-1}$ (Table 2). Not surprisingly, this is much lower than IP3 of the iron atom, which corresponds to the energy needed to convert the gaseous Fe(II) ion to Fe(III) (2957 kJ mol^{-1} ²⁶) but in hemoglobin the system is neutral. The calculated IP for hemoglobin is also significantly lower than IP1 of the isolated Fe atom (762 kJ mol^{-1} ²⁶), likely due to the coordination environment provided by the protein.

Next, we used PQ or a PQ derivative to bind to hemoglobin. As noted above, the binding mode was obtained from our previous docking study.¹⁷ The PQ derivatives considered in this study differ only in the substituent at the 5-position. We have considered three electron donating groups (EDGs), -OH , -CH_3 and -OCH_3 , and two electron withdrawing groups (EWGs), -NO_2 and -CF_3 . During geometry optimizations, all of the positively charged PQ derivatives transferred a proton to the carboxylic group of heme to form the $\text{-H}_2\text{N}\cdots\text{HOOC-}$ hydrogen bond interaction, which is what also occurred in our previous studies on 5-OH-

PQ.^{16, 17} With these derivatives bound, the ground states of the Fe(II) and Fe(III) systems were again in the quintet and quartet spin state, respectively. By comparing to the ground state energies, the energies needed to ionize hemoglobin could similarly be obtained and are listed in Table 2.

The energy needed to ionize Fe(II) was calculated to be the smallest for PQ bound to hemoglobin (603.8 kJ mol⁻¹), while the 5-NO₂-PQ system had the largest ionization energy. However, the energetic difference between these two is only 16 kJ mol⁻¹. In addition, the binding of the compounds other than PQ to hemoglobin even slightly increased the ionization energy. Therefore we concluded that the binding of PQ or one of its derivatives does not significantly lower the energy needed to ionize Fe(II) in hemoglobin to form methemoglobin. Furthermore, the higher ionization energy of Fe(II) in the 5-OH-PQ complex compared to that in the PQ complex does not match with the experimental finding^{14, 15} that 5-OH-PQ causes more methemoglobin formation than PQ. Hence, the mechanism that PQ and its derivatives cause a decrease of the ionization energy of hemoglobin to make it more easily able to be converted to methemoglobin is not feasible.

In summary, we considered in this study a third possible mechanism to explain the methemoglobinemia caused by the antimalarial drug primaquine. We hypothesized that the PQ derivatives do not directly participate in the electron transfer process, but rather simply bind to hemoglobin and lower hemoglobin's ionization potential. However, our results showed that the ionization potential of hemoglobin was not significantly lowered upon binding of different PQ derivatives to hemoglobin and hence this mechanism can be excluded. This further strengthens support for our previously proposed mechanism¹⁷ that a PQ metabolite increases methemoglobinemia formation by donating an electron to the hemoglobin-bound O₂ to facilitate its conversion to H₂O₂.

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Fig. 1.
The structures of primaquine (PQ) and 5-hydroxyprimaquine (5-OH-PQ).

Fig. 2.

A schematic illustration of the QM region used in this study. R represents the various substituents we have considered, i.e., $R = -\text{H}$, $-\text{CH}_3$, $-\text{OH}$, $-\text{OCH}_3$, $-\text{CF}_3$, and $-\text{NO}_2$.

Table 1

The relative energies (kJ mol^{-1}) of the complex at different multiplicities, without PQ or derivatives bound. The oxidation state of iron was set to be either +2 or +3

	Multiplicity	Relative energies
Fe(II)	Singlet	19.9
	Triplet	34.1
	Quintet	0.0
Fe(III)	Doublet	45.4
	Quartet	0.0
	Sextet	19.4

Table 2

The energies (ΔE , kJ mol⁻¹) needed to ionize Fe(II) in hemoglobin and in the complexes between hemoglobin and several PQ derivatives

	ΔE
Hemoglobin	606.4
Hemoglobin + PQ	603.8
Hemoglobin + 5-OH-PQ	619.7
Hemoglobin + 5-CH ₃ -PQ	611.9
Hemoglobin + 5-OCH ₃ -PQ	608.8
Hemoglobin + 5-NO ₂ -PQ	619.8
Hemoglobin + 5-CF ₃ -PQ	612.3