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Why does Sulfite Reductase employ Siroheme?

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Sulfite reductase (SiR) contains in the active site a unique assembly of siroheme and a [4Fe4S] cluster, linked by a cysteine residue. Siroheme is a doubly reduced variant of heme that is not used for the catalytic function in any other enzyme. We have used non-equilibrium Green's function methods coupled with density functional theory computations to explain why SiR employs siroheme rather than heme. The results show that direct, through vacuum, charge-transfer routes are inhibited when heme is replaced by siroheme. This ensures more efficient channelling of the electrons to the catalytic iron during the six-electron reduction of sulfite to sulfide, limiting potential side-reactions that could incur if the incoming electrons were delocalized onto the macrocyclic ring.

The active site of sulfite reductase (SiR) comprises an unusual assembly of two directly connected cofactors (cf. Figure 1): a siroheme group, which binds the substrate, and a cubane Fe_4S_4 cluster, which acts as a molecular pump that transfers to siroheme electrons provided by nearby flavoproteins.[1] Siroheme is a modified version of heme belonging to the same isobacteriochlorin class. It differs from heme in that two of the pyrrole rings are partially saturated (cf. Figure 2). This changes the nature of the π-system and rings C and D are no longer planar (see right side of Figure 1). The cubane cofactor is engulfed inside the active site pocket, while the siroheme is equatorially exposed to the surface with the partially saturated rings oriented towards the solvent.[2]

Interestingly, although heme and cubane groups are known to be simultaneously used by some enzymes,[3] the two cofactors are never covalently connected to each other directly – with the exception of the SiR active site, where a cysteine thiolate bridges one cubane Fe ion to the siroheme. Conversely, siroheme is never present alone in any enzyme active site (besides in enzymes involved in its own biosynthesis) – it is always coupled to a cubane iron–sulfur cluster.[4] While the prime role of the cubane in the SiR mechanism is to provide electrons for the reaction (six electrons are needed to reduce sulfite to S^{2-}), the choice of siroheme vs. heme in SiR has not been rationalized. Structural models of the siroheme–cubane site of SiR have been synthesized, but employing heme rather than siroheme. Initially,[5] these models showed no catalytic activity, but more recent versions tuning the second-sphere interaction of the two cofactors with elements from the native enzyme were shown to possess catalytic activity.[6] This further emphasizes the question why SiR uses siroheme rather than heme.

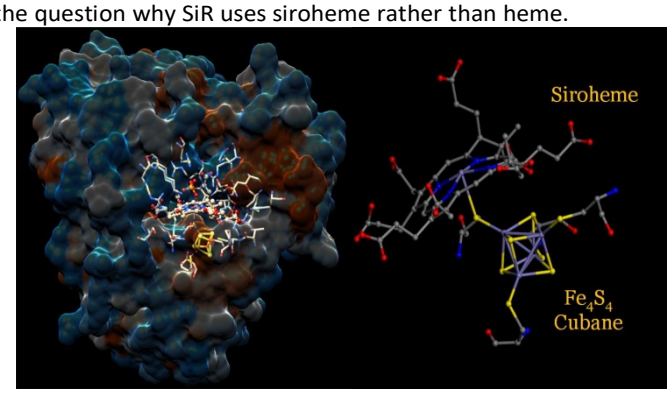

Figure 1. Left: The structure of sulfite reductase (pdb entry 1AOP) with hydrophilic areas of its surface shaded in blue and hydrophobic areas in red. The active site is represented with balls and sticks and its surrounding residues with sticks. Right: Close view of the active site comprised of siroheme and the Fe_4S_4 cubane cluster. Fe is represented in violet, N in blue, S in yellow, O in red and C in grey. Hydrogen atoms are omitted for clarity.

In this investigation, we study how siroheme modifies the electron-transfer properties of the SiR active site compared to heme by using computational methods, providing a plausible explanation why SiR uses siroheme rather than heme.

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Electronic Supplementary Information (ESI) available: Models and methods, the donor-acceptor and molecular junction analysis together with the extended analysis of different (siro)heme-cubane structures are provided in the Supporting Information section.) See DOI: 10.1039/x0xx00000x

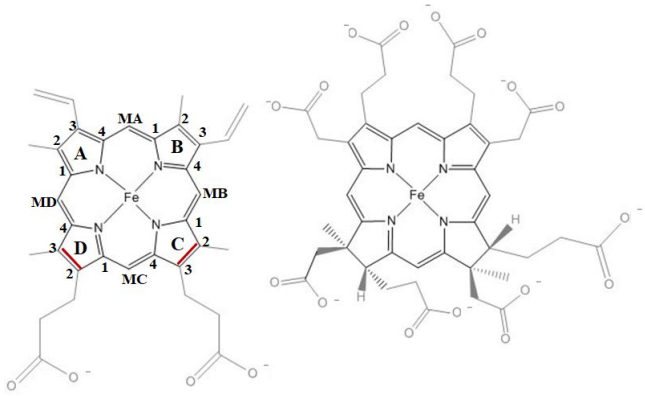

Figure 2. Structures of heme *b* (left) and siroheme (right). The peripheral substituents are shaded in grey. The extra two double bonds present in the heme ring are highlighted in red.

By treating the SiR active site as a molecular junction (cf. Figure 3), the non-equilibrium Green's function coupled with density functional theory (NEGF-DFT) framework can be employed to compute its electron-transport properties.[7] The computed electron conductance is directly connected to the rate constant of the electron transfer process[8] and thus provides insights on the kinetic aspects of the reaction (further theoretical details are provided in the Supporting Information).

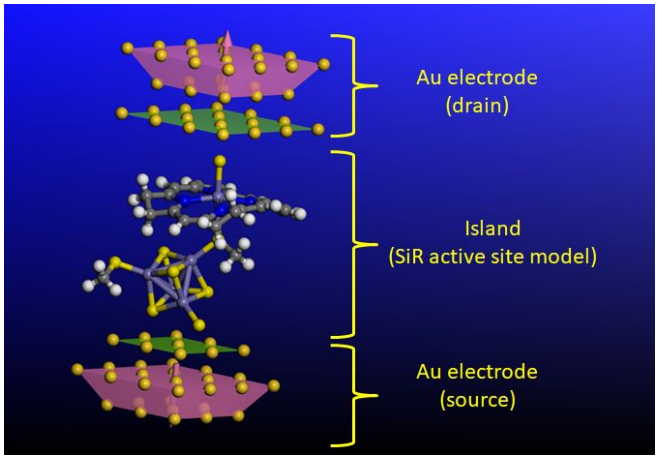

Figure 3. SiR active site as a molecular junctions connecting two Au electrodes. Au atoms are depicted in dark yellow, Fe in violet, N in blue, S in yellow, O in red, C in grey and H in white.

Using this approach, an electron-route analysis was performed on four routes by which electrons can be transferred from the cubane to the (siro)heme cofactor. The first route deals with the charge transfer trough the bridging cysteinate sulfur atom (S_{bridge}), passing from the cubane iron that is involved in the interfactor bond (Fe_1) to the (siro)heme Fe ion (Fe_{heme}). The other three routes entail direct, through space[9], charge transfer to Fe_{heme} via the porphyrin ring from the other three atoms of the cubane side facing (siro)heme (cf. Figure 4). Both bridged and direct routes comprise two steps. In the bridged route the first step is represented by the cubane $Fe_1 \rightarrow S_{bridge}$ electron transfer and the second step by the $S_{bridge} \rightarrow Fe_{heme}$ transfer. In the direct routes the first step is represented by the cubane $Fe_2 \rightarrow$porphyrin electron transfer and the second step by the porphyrin$\rightarrow Fe_{heme}$ transfer. The bridged and direct routes differ in terms of location of the transient radical character generated by the transmitted electron: in the former case, the transient radical character is on the S_{bridge}, while in the latter case it is on the porphyrin ring.

The bridging route passes through two bonds, Fe_1–S_{bridge} and S_{bridge}–Fe_{heme} and in both models, the conductance is higher in the first than in the second (cf.

Table 1). For the other three routes, involving direct (through space) charge transfer between the two cofactors, different paths were considered from each cubane atom to its closest porphyrin C atoms for each path.

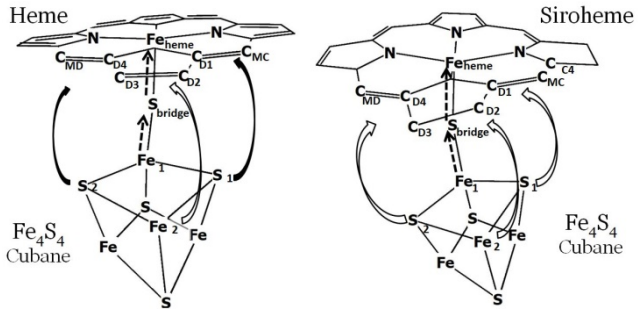

Figure 4. Electron routes investigated in the heme–cubane (left) and siroheme–cubane systems (right). The bridged route is depicted with dashed arrows, while the direct routes are shown with solid arrows. High conductance is depicted in black, while low conductance is in white. The structures were optimised at the B3LYP-D3/def2-TZVP level of theory before the electron-route analysis was performed (alternative structures are discussed in the SI).

Compared to heme, siroheme slightly decreases the conductance of the first step of the bridged route and slightly increases it in the second step. On the other hand, for the three direct routes, the conductance is appreciably lower for siroheme than for heme. While the $Fe_2 \rightarrow$porphyrin conductance remains virtually unchanged when exchanging heme by siroheme, the routes starting from the sulfur atoms are significantly inhibited. By considering all the possible paths of each direct route this effect becomes even clearer: the average conductance in the direct routes drops from a total value of 0.7 a.u. in the heme variant to 0.1 a.u. when siroheme is used.

As can be seen in

Table 1, there is no correlation between the distance of two atoms and transmission value. The difference between heme and siroheme in terms of conductance derives from the phase of the orbitals involved in the direct routes. Notably, the involved carbon atoms on the siroheme ring are sp^3 hybridized, whereas in heme they are part of the conjugated π system (i.e. sp^2 hybridized). By saturating the two double bonds involved in the direct routes, siroheme interrupts the porphyrin π system. This interruption causes the porphyrin orbitals to interact with the cubane orbitals involved in the direct routes in a less efficient manner.

The results of the computed conductance reveal that the bridged route is always more favourable than the direct routes. This is in accordance with the well-known fact that quantum tunnelling-driven charge transfer in biological systems occur over longer distances (~14 Å)[10] when the tunnelling goes through the amino acids rather than when passing through vacuum.[11] Although the conductance in the through-bond route is slightly altered when siroheme replaces heme, a much more remarkable decrease is seen for the conductance through the direct routes. This suggests that siroheme inhibits the electron transfer via the edge of the porphyrin. Avoiding these routes probably assures that the porphyrin is kept in a radical-free state, thereby reducing the risk of unwanted side-reactions such as:

- *Sulfheme formation:* lysine and arginine residues form a low-polarity environment around the Fe_{heme} distal position. Such environments are known[12] to initiate sulfheme formation reaction in myoglobin and hemoglobin in the presence of H_2S. Furthermore, heme radical states are invoked[13] in the sulfheme formation mechanism. In a different mechanism,[14] H_2S was shown to react with heme via HS^-, i.e. the intermediate present in the last steps of SiR's catalytic cycle.
- *Heme–solvent reactions:* being exposed to the solvent with the side involved in the direct routes, the transient heme radical could be susceptible to reacting with the solvent molecules.
- *Heme-intermediate reactions:* SiR catalyses sulfite reduction via intermediates comprising also positively charged adducts that can react with the transient negatively charged heme.[15]

Sulfheme formation in hemoglobin and myoglobin is known[16] to drastically decrease the Fe_{heme} affinity for the substrate. Similarly, the formation of this species in SiR is expected to affect the substrate binding to Fe_{heme}, an undesirable event considering the rapid six-electron reduction that the enzyme needs to undergo. Nevertheless, the purpose of SiR implies dissociation of the H_2S product at the end of the catalytic cycle and not its storage at the heme periphery (like hemeproteins that store H_2S in the sulfheme form and use it for signalling).[12] Reaction of a heme radical with water solvent can easily lead to the formation of hydroxyheme. This species is known[17] to be present in heme oxygenase's heme-degrading mechanism. Also, the displacement of an intermediate on the heme ring can drastically disturb the catalytic cycle and produce undesired products.

In the heme–cubane variant, the second steps of the direct routes (porphyrin→Fe_{heme}) have a lower conductance than the first steps (average 0.2 vs. 0.7 a.u. respectively) and, more important, a lower conductance than the second step of the bridged route (i.e. S_{bridge}→Fe_{heme}). The low conductance of the porphyrin→Fe_{heme} steps suggests that, once on the porphyrin, the electron delocalizes in it and the transfer to Fe_{heme} is delayed. This emphasises that, although in the first step the direct route matches the corresponding bridged step, overall the bridged route is more efficient in transmitting electrons from the cubane to the Fe_{heme}. Thus, by inhibiting the porphyrin→Fe_{heme} step, SiR avoids the futile delocalization of the transmitted electron onto the macrocycle, a delocalization that would hinder the substrate-reduction process.

Table 1. Computed conductance (*G*) for the investigated routes in the (siro)heme–cubane systems. Atom numbers are given in Figure 4; *d* represents the distance (in Å) between the two atoms. For the direct routes, the conductance of the second step (i.e. porphyrin→Fe_{heme}) is given in parenthesis.

Route	Heme–cubane				Siroheme–cubane			
	Atoms		*d*	*G*	Atoms		*d*	*G*
	#1	#2	(Å)	*a.u.*	#1	#2	(Å)	*a.u.*
bridged	Fe_1	S_{bridge}	2.4	**2.0**	Fe_1	S_{bridge}	2.3	**1.9**
	S_{bridge}	Fe_{heme}	2.2	**0.7**	S_{bridge}	Fe_{heme}	2.4	**1.0**
direct	S_1	C_{MC}	3.4	0.9(0.4)	S_2	C_{C4}	3.6	0.2(0.4)
		C_{D1}	3.3	1.3(0.1)		C_{MC}	3.2	0.1(0.1)
		C_{D2}	3.4	1.5(0.2)		C_{D1}	3.4	0.1(0.1)
	Average			**1.2(0.2)**	Average			**0.1(0.2)**
direct	S_2	C_{D3}	3.4	0.9(0.2)	S_1	C_{D3}	3.7	0.3(0.2)
		C_{D4}	3.4	0.9(0.1)		C_{D4}	3.7	0.1(0.1)
		C_{MD}	3.4	0.3(0.4)		C_{MC}	4.0	0.2(0.4)
	Average			**0.7(0.2)**	Average			**0.2(0.2)**
direct	Fe_2	C_{D2}	3.7	0.1(0.2)	Fe_2	C_{D2}	4.0	0.2(0.2)
		C_{D3}	3.7	0.2(0.2)		C_{D3}	3.9	0.1(0.2)
	Average			**0.1(0.2)**	Average			**0.1(0.2)**
Average direct routes				**0.7(0.2)**				**0.2(0.2)**

In conclusion, siroheme tunes the electron transfer from the cubane cofactor to the substrate such that, when compared to the heme variant of the SiR active site, the states associated with the through-vacuum charge transfer are inhibited, while the states involved in the through-bridge charge transfer are modified to increase the electron transmission. Thus, the role of siroheme is to block the delaying porphyrin→Fe_{heme} step in order to increase the overall charge transfer from the cubane cofactor. Furthermore, siroheme reduces the risk of porphyrin acquiring partial radical character that comes as an effect of the electrons being transmitted from the cubane via routes that involve the periphery of the porphyrin π-system. By avoiding

these charge-transfer channels, the macrocycle is protected against undesired radical attack.

Conflicts of interest

There are no conflicts to declare.

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