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Why does sulfite reductase employ siroheme?†

Adrian M. V. Brânzanic, [ab] Ulf Ryde *[c] and Radu Silaghi-Dumitrescu *[a]

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 a 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 occur 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.* Fig. 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.* Fig. 2). This changes the nature of the π-system and rings C and D are no longer planar (see right side of Fig. 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 of why SiR uses siroheme rather than heme.

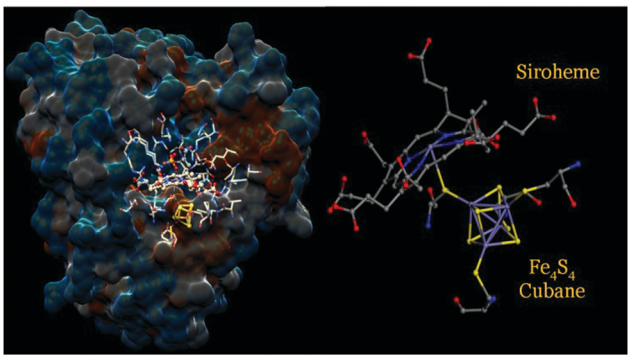

Fig. 1 Left: The structure of sulfite reductase (pdb entry 1AOP) with hydrophilic areas of its surface shaded in blue and hydrophobic areas in red. The active site is represented with balls and sticks and its surrounding residues with sticks. Right: Close view of the active site composed of siroheme 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 of why SiR uses siroheme rather than heme.

By treating the SiR active site as a molecular junction (*cf.* Fig. 3), the non-equilibrium Green's function coupled with a

[a] *Department of Chemistry, Babes-Bolyai University, Cluj-Napoca, Romania. E-mail: rsilaghi@chem.ubbcluj.ro*
[b] *Institute of Interdisciplinary Research in Bio-Nano-Sciences, Babes-Bolyai University, Cluj-Napoca, Romania*
[c] *Department of Theoretical Chemistry, Lund University, Lund, Sweden*

† 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. See DOI: 10.1039/c9cc05271b

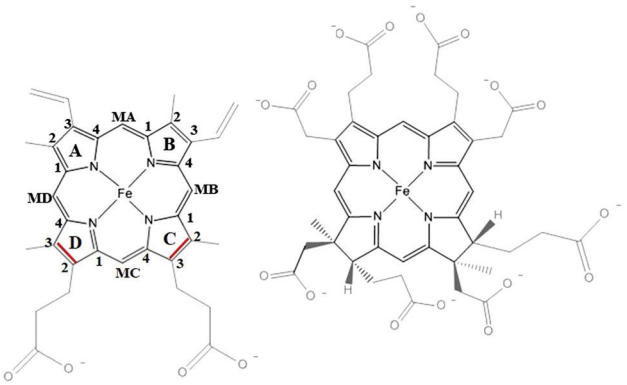

Fig. 2 Structures of heme *b* (left) and siroheme (right). The peripheral substituents are shaded in grey. The extra two double bonds present in the heme ring are highlighted in red.

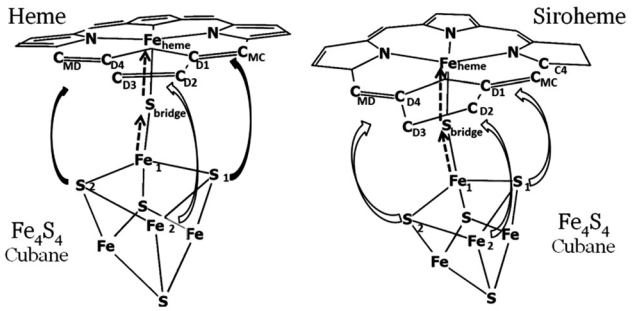

Fig. 4 Electron routes investigated in the heme–cubane (left) and siroheme–cubane systems (right). The bridged route is depicted with dashed arrows, while the direct routes are shown with solid arrows. High conductance is depicted in black, while low conductance is in white. The structures were optimised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

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 into the kinetic aspects of the reaction (further theoretical details are provided in the ESI†).

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 through 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.* Fig. 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.

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.

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.

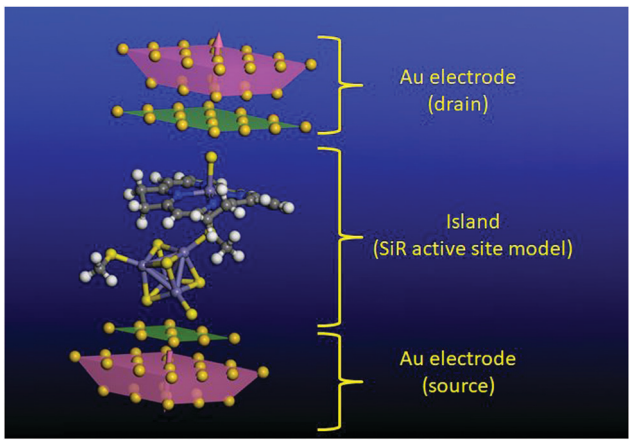

Fig. 3 SiR active site as a molecular junction connecting two Au electrodes. Au atoms are depicted in dark yellow, Fe in violet, N in blue, S in yellow, O in red, C in grey and H in white.

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

	Heme–cubane				Siroheme–cubane			
	Atoms				Atoms			
Route	#1	#2	*d* (Å)	*G* (a.u.)	#1	#2	*d* (Å)	*G* (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_1	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)
Direct	S_2	C_{D3}	3.4	0.9(0.2)	S_2	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)
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)

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 occurs over longer distances ($\sim$14 Å)[10] when the tunnelling goes through the amino acids rather than when passing through a 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.

It is well-known that radicals are more reactive than closed-shell molecules. Therefore, it is likely that a radical character of the porphyrin ring, caused by through-space electron transfer, may increase the risk of unwanted side reactions. There are several examples of such reactions involving positively charged heme radicals (*i.e.* after removal of one electron), *e.g.* sulfheme formation[12] and heme-solvent reactions,[13] and it is likely that similar reactions can also be caused by negatively charged heme radicals. Furthermore, in the case of heme-solvent reaction, it is known that surrogate electron donors (*e.g.* ascorbic acid or NADPH) are needed in order to produce hydroxyheme species.[14] In the case of the heme–cubane system, the electrons that facilitate the hydroxyheme formation would be provided *via* direct routes by the endogenous Fe_4S_4 cluster. Lastly, the positively charged intermediates present in the SiR catalytic cycle[15] could easily react with the negatively charged heme species.

In the heme–cubane variant, the second steps of the direct routes (porphyrin $\rightarrow Fe_{heme}$) have a lower conductance than the first steps and, more importantly, a lower conductance than the second step of the bridged route (*i.e.* $S_{bridge} \rightarrow Fe_{heme}$). The low conductance of the porphyrin $\rightarrow Fe_{heme}$ steps suggests that, once on the porphyrin, the electron delocalizes in it and the transfer to Fe_{heme} is delayed. Indeed, it is known that heme systems prefer to transfer electrons across the porphyrin *via* routes that avoid the Fe center.[16] 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 $\rightarrow Fe_{heme}$ step, SiR avoids the futile delocalization of the transmitted electron onto the macrocycle, a delocalization that would hinder the substrate-reduction process.

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 $\rightarrow Fe_{heme}$ 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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