LETTER

Redox potentials in the decaheme cytochrome MtrF: Poisson–Boltzmann vs. molecular dynamics simulations

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We previously computed the redox potentials for the 10 hemes in the decaheme cytochrome MtrF using thermodynamic integration (TI) in combination with all-atom, explicit solvent molecular dynamics (MD) simulation (1). In a recent study, Watanabe et al. (2) recomputed these potentials using a Poisson–Boltzmann (PB) continuum approach. The potentials obtained from MD for the all-oxidized protein gave a nearly symmetrical free energy profile along the octaheme chain with a small overall driving force of -48 ± 66 meV from heme 10 to heme 5 and two symmetrical free energy maxima of ~200 meV at heme 9 (domain IV) and heme 4 (domain II). The PB equation gave a slightly larger overall driving force of −118 meV and predicted a free energy maximum in domain IV as well. However, by contrast to TI, a mostly downward slope through the rest of the chain was observed (i.e., no second maximum in domain II).

Watanabe et al. (2) rationalize the asymmetry of their profile by noting that it is "mainly caused by the acidic residues at Asp631, Asp518, Asp490 (in domain IV)... . These acidic residues are not present in the corresponding regions of domain II." This argument cannot be correct because the authors show that protonation of Asp631 (most important residue according to tables 2 and 3 in ref. 2) leaves the qualitative features of the profile unchanged. Their apparent electron sink in domain II remains unexplained.

Watanabe et al. (2) criticize our reported residue electrostatic contributions as being too high. However, this ignores the fact that, in MD, these are the bare electrostatic contributions that, when added up over all residues and the solvent, give the full, thermally averaged electrostatic potential at the heme site. By contrast, in the PB equation, the residue contributions are screened by a simplistic dielectric medium used to approximate the protein environment. Therefore, it is only meaningful to compare the sign but not the magnitude of the single-residue contributions.

Finally, Watanabe et al. (2) attempted to reproduce our TI/MD redox potentials, but none of their profiles matched ours, concluding that this "argues against the quality of their [Breuer et al.'s] calculated E_m values." However, close inspection of their TI protocol raises serious concerns. "TI simulations were conducted over 10 ns with an MD time step of 2.0 fs, namely $\Delta\lambda = 2.0 \times 10^{-7}$...oxidized heme ($Fe^{3+}$) was gradually reduced (to Fe^{2+}) over 10 ns." Apparently, in their approach, the TI coupling parameter λ was erroneously changed every MD integration time step. This corresponds to a single configuration being used to define an ensemble average, which is nonsensical. This flaw in their protocol seems to be a much more likely cause for the different TI-derived free energy profiles reported in figure 5 of ref. 2 than the supposed slow structural fluctuations in MtrF; for these fluctuations, the authors do not provide any evidence, and they do not seem plausible given the considerable stiffness of the decaheme motif in MtrF.

1 Breuer M, Zarzycki P, Blumberger J, Rosso KM (2012) Thermodynamics of electron flow in the bacterial deca-heme cytochrome MtrF. *J Am Chem Soc* 134:9868–9871.

2 Watanabe HC, Yamashita Y, Ishikita H (2017) Electron transfer pathways in a multiheme cytochrome MtrF. *Proc Natl Acad Sci USA* 114:2916–2921.

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