Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Insights into the Mechanism of Oxidation of Dihydroorotate to Orotate in Human Class 2 Dihydroorotate Dehydrogenase: A QM/MM Free Energy Study

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Supplementary Information

1. Correction of SCC-DFTB/MM by B3LYP(MP2)/MM method

To correct the possible inaccuracies in the SCC-DFTB semiempirical method and verify its reliability with higher theoretical levels (i.e. DFT or MP2). In this sense, we performed out single-point B3LYP-D3/MM and MP2/MM calculations using representative snapshots from MC, INT and TS states, which are the same used on the Interaction Energy Decomposition analysis. The corrected SCC-DFTB/MM results based on the B3LYP-D3/MM and MP2/MM calculations improve not only the QM level as well as the MM interactions and its polarization on the QM subsystem[1]. The SCC-DFTB correction was performed using a straightforward one-step free energy perturbation,

$$\Delta G_{\mathrm{B3LYP(MP2)-DFTB}} = -k\mathrm{T}\ln\langle e^{-\beta(\mathrm{E_{B3LYP(MP2)/MM}-E_{DFTB/MM}})}\rangle_{\mathrm{DFTB/MM}}$$

which was done at both MC and INT (and P) states to correct the ΔG° for the deprotonation and hydride equivalent steps, respectively. Besides, the $\Delta G^{\ddagger}$ was corrected for the hydride equivalent step, using INT and P states. The difference between the perturbative corrections at these states gives the B3LYP-D3/MM and MP2/MM corrections to the free energy of the oxidation reaction catalyzed by human DHOD. The B3LYP(MP2)/6-31G* was applied in order to perform the corrections. The ΔG° obtained by SCC-DFTB/MM method for the deprotonation and hydride equivalent steps is estimated at –6.54 and –13.50 kcal·mol^{-1}, respectively. Whereas the ΔG° calculated by B3LYP-D3/MM is –14.75 and –15.15 kcal·mol^{-1}, respectively; and the ΔG° calculated by MP2/MM is –15.78 and –17.18 kcal·mol^{-1}, respectively. The $\Delta G^{\ddagger}$ obtained by SCC-DFTB/MM method for the hydride equivalent step is 2.04 kcal·mol^{-1}, and the $\Delta G^{\ddagger}$ calculated by B3LYP-D3/MM and MP2/MM are 10.84 and 7.55 kcal·mol^{-1}, respectively. The corrected calculations show the qualitative reliability of the semiempirical method (SCC-DFTB) to the high hamiltonian (B3LYP-D3 or MP2). Besides, the same approach was successfully applied to elucidate the catalytic mechanism for the DHOD from *L. lactis*[2].

1. D. Riccardi, P. Schaefer and Q. Cui, *Journal of Physical Chemistry B*, 2005, 109, 17715-17733.
2. J. R. A. Silva, A. E. Roitberg and C. N. Alves, *Journal of Physical Chemistry B*, 2015, 119, 1468–1473.