Supporting Information

Mechanistic Investigation of Isonitrile Formation Catalyzed by the Non-Heme Iron/α-KG Dependent Decarboxylase (ScoE)

Hong Li, Yongjun Liu*

Key Lab of Colloid and Interface Chemistry, Ministry of Education, School of Chemistry and Chemical Engineering, Shandong University, Jinan, Shandong 250100, China

(*Corresponding Author: Tel.: +86 53188365576; fax: +86 53188564464. Email address: yongjunliu_1@sdu.edu.cn)

Table S1. Relative energies of optimized reactant models (R_{23} , R_{24} and R_{25}) at the triplet and quintet states. The relative energies are given in kcal/mol. The energy barriers of H_{5a} -abstraction at the quintet state are also listed for comparison.

| | Triplet | Quintet | Septet | Barrier |
|-------|---------|---------|--------|---------|
| 23 ns | 4.9 | 0 | 18.4 | 20.8 |
| 24 ns | 5.5 | 0 | 15.9 | 20.1 |
| 25 ns | 6.6 | 0 | 13.9 | 17.6 |

Table S2. Key bond lengths of optimized structures at triplet and quintet spin states involved in the desaturation. All distances are given in angstroms. The data at quintet state are listed in parentheses.

| | | | U | U | | | 1 | | | | |
|-------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| | r1 | r2 | r3 | r4 | r5 | r6 | r7 | r8 | r9 | r10 | r11 |
| R | 1.64(1.64) | 1.97(2.13) | 2.00(2.13) | 2.06(2.03) | 2.09(2.21) | 1.94(1.95) | 1.86(1.85) | 2.11(2.10) | 1.85(1.86) | 1.71(1.72) | 1.66(1.66) |
| TS1 | 1.77(1.74) | 1.98(2.17) | 2.00(2.05) | 2.04(2.03) | 2.09(2.40) | 1.94(1.95) | 1.87(1.85) | 1.95(1.92) | 2.02(2.14) | 1.76(1.74) | 1.66(1.65) |
| IM1 | 1.74(1.83) | 1.99(2.19) | 1.98(2.00) | 2.01(1.99) | 2.06(2.40) | 1.95(1.94) | 1.89(1.96) | 2.01(2.00) | 1.97(1.98) | 1.68(1.68) | 1.66(1.66) |
| TS2 | 1.94(1.86) | 1.96(2.15) | 1.98(2.01) | 1.99(2.01) | 2.04(2.41) | 1.96(1.96) | 2.19(2.14) | 2.12(2.07) | 1.87(1.92) | 1.62(1.65) | 1.69(1.68) |
| IM2 | 2.04(2.23) | 2.18(2.08) | 2.02(2.06) | 1.98(2.15) | 3.14(2.96) | 2.03(2.04) | 2.97(2.81) | 2.25(2.14) | 1.94(2.00) | 1.74(1.75) | 1.71(1.70) |
| | r1 | r2 | r3 | r4 | r5 | r6 | r7 | r8 | r9 | r10 | rll |
| TS1_N | 1.76(1.73) | 2.01(2.08) | 1.99(2.22) | 2.10(2.09) | 2.11(2.17) | 2.00(2.16) | 1.79(1.95) | 2.06(2.03) | 2.08(2.08) | 1.79(1.81) | 1.67(1.67) |
| IM1_N | 1.83(1.82) | 1.99(2.08) | 1.98(2.26) | 2.03(2.02) | 2.06(2.09) | 1.96(2.01) | 1.85(1.93) | 2.05(2.03) | 1.98(2.00) | 1.96(1.77) | 1.67(1.64) |
| TS2_N | 1.88(1.92) | 1.96(2.16) | 1.99(2.21) | 1.97(2.16) | 2.18(2.21) | 1.92(1.94) | 1.90(1.83) | 2.03(2.05) | 1.77(1.92) | 1.77(1.80) | 1.68(1.66) |
| IM2_N | 2.02(2.29) | 2.29(2.23) | 1.98(2.17) | 1.97(2.16) | 3.10(2.43) | 1.98(2.01) | 1.86(2.26) | 2.38(2.18) | 1.91(1.78) | 1.86(2.01) | 1.67(1.67) |

| | ⁵ IM2_N | ⁵ TS2_N | ⁵ IM1_N | ⁵ TS1_N | (3)5{7}R | ⁵ TS1 | ⁵ IM1 | ⁵ TS2 | ⁵ IM2 |
|-------|--------------------|--------------------|--------------------|--------------------|--------------------|------------------|------------------|------------------|------------------|
| QM-I | -34.3 | 16.9 | -2.3 | 9.8 | (6.6)0.0{13.9} | 17.6 | -5.4 | 5.7 | -34.8 |
| QM-II | -34.1 | 18.3 | -3.6 | 7.5 | 7.5 (6.3)0.0{13.7} | | -5.8 | 7.1 | -35.8 |
| | | | | | | | | | |
| | ⁵ IM2' | ⁵ TS3 | ⁵ IM3 | ⁵ TS4 | ⁵ P | ⁵ TS4 | rebound | ⁵ II | M4 |
| QM-I | 0 | 9.8 | -4.9 | 2.6 | -30.6 | 3.2 | | -5. | 3.4 |
| QM-II | 0 | 9.9 | -5.1 | 3.2 | -33.8 | 4.5 | | -54 | 4.8 |

Table S3. Relative energies (kcal/mol) of involved species in the desaturation and decarboxylation, as well as rebound reaction using two different QM regions. The energy of ⁵R was set to zero.

Table S4. Calculated spin densities of key atoms and groups at triplet and quintet spin states involved in the desaturation. The data at quintet state are listed in parentheses.

| | Fe | O _{Fe} | Fe-O | C ₅ | Ν | substrate |
|-------|------------|-----------------|------------|----------------|------------|------------|
| R | 1.45(3.22) | 0.58(0.46) | 2.03(3.68) | 0(0) | 0(0) | 0(0) |
| TS1 | 1.02(2.84) | 0.51(0.36) | 1.53(3.20) | 0.33(0.19) | 0.06(0.26) | 048(0.57) |
| IM1 | 0.98(2.76) | 0.04(0.03) | 1.02(2.79) | 0.58(0.58) | 0.23(0.24) | 0.99(0.99) |
| TS2 | 1.78(3.00) | 0(0.02) | 1.78(3.02) | 0.10(0.51) | 0.2(0.19) | 0.14(0.84) |
| IM2 | 1.92(3.67) | 0(0.03) | 1.92(3.70) | 0(0) | 0(0) | 0(0) |
| | Fe | O _{Fe} | Fe-O | C ₅ | Ν | substrate |
| TS1_N | 0.94(2.73) | 0.26(0.13) | 1.20(2.86) | 0.01(0.01) | 0.53(0.67) | 0.80(0.99) |
| IM1_N | 0.97(2.75) | 0.05(0.02) | 1.02(2.77) | 0(0) | 0.78(0.78) | 0.99(0.99) |
| TS2_N | 1.06(2.86) | 0.04(0.06) | 1.10(2.92) | 0.09(0.02) | 0.62(0.87) | 0.94(1.00) |
| IM2_N | 1.98(3.74) | 0(0.03) | 1.98(3.77) | 0(0) | 0(0) | 0(0) |

Table S5. Key bond lengths of optimized structures at triplet and quintet spin states involved in the decarboxylation and hydroxylation reaction. All distances are given in angstroms. The data at quintet state are listed in parentheses.

| | r1 | r2 | r3 | r4 | r5 | r6 | r7 | r8 | r9 | r10 | r11 |
|------------------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| IM2' | 1.62(1.63) | 1.97(2.14) | 1.98(2.08) | 2.05(2.03) | 2.12(2.28) | 1.94(1.96) | 1.83(1.81) | 2.21(2.11) | 1.99(2.01) | 1.76(1.76) | 1.60(1.60) |
| TS3 | 1.73(1.73) | 1.98(2.16) | 1.99(2.08) | 2.06(2.04) | 2.11(2.32) | 1.95(1.96) | 1.81(1.79) | 2.04(2.05) | 2.11(2.25) | 1.81(1.82) | 1.61(1.61) |
| IM3 | 1.82(1.82) | 2.17(2.17) | 2.01(2.02) | 2.00(2.01) | 2.40(2.40) | 1.95(1.95) | 1.92(1.91) | 2.13(2.13) | 2.02(2.03) | 1.79(1.79) | 1.61(1.61) |
| TS4 | 1.82(1.82) | 2.19(2.18) | 2.02(2.03) | 2.00(2.02) | 2.40(2.39) | 1.97(1.96) | 1.87(1.89) | 2.29(2.20) | 2.04(2.07) | 1.84(1.81) | 1.61(1.61) |
| Р | 2.04(2.17) | 2.33(2.24) | 2.02(2.20) | 2.00(2.17) | 2.69(2.41) | 2.04(2.09) | 1.01(1.01) | 4.11(4.17) | 3.68(3.69) | 2.19(2.18) | 1.63(1.63) |
| TS4 _{rebound} | 1.84(1.84) | 2.15(2.16) | 2.04(2.04) | 2.03(2.05) | 2.40(2.42) | 1.96(1.96) | 1.84(1.92) | 1.94(2.03) | 2.12(2.08) | 1.83(1.87) | 1.61(1.61) |
| IM4 | 3.02(2.93) | 2.08(2.11) | 2.03(2.26) | 2.08(2.11) | 2.37(2.25) | 1.99(2.06) | 2.98(2.96) | 3.17(3.14) | 1.99(1.99) | 1.75(1.76) | 1.59(1.59) |

| | 5 | 1 | | 1 | | |
|------|------------|-----------------|------------|----------------|------------|------------|
| | Fe | O _{Fe} | Fe-O | C ₅ | Ν | substrate |
| IM2' | 1.37(3.16) | 0.64(0.53) | 2.01(3.69) | 0(0) | 0(0) | 0(0) |
| TS3 | 1.03(2.89) | 0.53(0.37) | 1.56(3.26) | 0.13(0.11) | 0.14(0.17) | 0.45(0.50) |
| IM3 | 1.01(2.76) | 0.06(0.04) | 1.07(2.80) | 0.41(0.41) | 0.25(0.27) | 0.96(0.95) |
| TS4 | 1.04(2.88) | 0.06(0.01) | 1.10(2.89) | 0.04(0.29) | 0.14(0.26) | 0.91(0.93) |
| Р | 1.95(3.67) | 0(0.07) | 1.95(3.74) | 0(0) | 0(0) | 0(0) |

Table S6. Calculated spin densities of key atoms and groups at triplet and quintet spin states involved in the decarboxylation. The data at quintet state are listed in parentheses.



Figure S1. Overlap of the active sites of ScoE enzyme (PDB ID 6DCH, wheat color) and TauD (PDB ID 10S7, blue color).



Figure S2. Two representative conformations A and B obtained from the docking results based on the criterion of RMSD of 2.0 Å. All distances are given in angstroms.

Docking scheme: during the docking process, three-dimensional grid map was generated in the active site, which was set to 30 Å \times 30 Å \times 30 Å with a grid spacing of 0.375 Å. The protein was kept rigid. Then fifty independent docking runs were carried out.



Figure S3. (a) Constructed solvation model for MD simulations; (b) RMSDs for the backbone atoms of the ScoE enzyme-substrate complex in 25-ns MD simulations.

During the solvation setup, the protein was firstly solvated into a larger box of pre-equilibrated waters. Next, all the added water molecules that locate within 2.5 Å from the heavy protein atoms were removed. Then the size of the solvent box was set, in which the distance between the protein edge and the water box edge was set to 6 Å. Subsequently, the longest axis was set to be the spherical diameter, and those water molecules that outside the sphere were removed. Finally, a quick energy minimization was done with the protein and ligand fixed in their energy minimized positions.



Figure S4. Overlap of optimized active site structures of R_{23} , R_{24} and R_{25} . For clarity, the QM region is shown in sticks.



Figure S5. Optimized structure of R-B at the quintet state.



Figure S6. Optimized reactant structures of R at triplet and quintet spin states. Coordination bond lengths (r1-r6) and hydrogen bond lengths (r7-r11) are also shown here. All distances are given in angstroms.



Figure S7. (a) Succ-I/Succ-II: two models with different poses of succinate; (b) optimized structure (R') by using Succ-I/Succ-II models. For comparison, the initial constructed enzyme-substrate complex (c) and the structure of R (d) are also presented here. The relative energies are given in kcal/mol.



Figure S8. Optimized structures and relative energies (kcal/mol) of involved species in H-abstraction with B3LYP-D3 at the quintet state. The parameters with B3LYP-D3 are marked with asterisk. The energies of reactants at the quintet state were set to zero.



Figure S9. Energy profiles (kcal/mol) for path- H_{5a} and path- H_N at the triplet spin state. The relative energy of ⁵R is set to zero. Energy profile for the hydroxylation reaction is also presented here.



Figure S10. Optimized structures and relative energies of intermediates and transition states involved in the σ - and π -pathways at the quintet state. The data involved in the π -pathway are listed in parentheses, while those of the σ -pathway are marked with subscript σ .



Figure S11. Possible reaction pathways based on the hydroxylated intermediate (IM2_{rebound}).



Figure S12. Energy profiles (kcal/mol) for decarboxylation and hydroxylation reaction at triplet spin state. The relative energy of ⁵IM2' is set to zero.



Figure S13. (a) Optimized structure of $IM2_N'$ at the quintet state; (b) Energy profile of the decarboxylation reaction by using $IM2_N'$ model.



Figure S14. Electron-shift diagrams and valence electron orbitals of ⁵TS4 and ⁵P.



Figure S15. Scanned energy profile for dehydration-assisted decarboxylation using $RC1=[r1(H_1-O_{Fe})-r2(C_5-O_{Fe})-r3(C_5-C_6)]$ as the reaction coordinate at the B1 level. The scanning starts from ⁵IM4. All distances are shown in angstroms and the relative energies in kcal/mol.



Figure S16. Scanned energy profile for dehydration-assisted decarboxylation using $RC2=[r1(H_3-O_{Fe})-r2(C_5-O_{Fe})-r3(C_5-C_6)]$ as the reaction coordinate at the B1 level. Because residue Y96 acts as proton source, another computational model (IM4') was constructed, in which the substrate rotates 180° compared to the orientation of the substrate in IM4. All distances are shown in angstroms and the relative energies in kcal/mol.



Figure S17. Energy profile (kcal/mol) for the decarboxylation at quintet spin state for R310L mutant. The relative energy of ${}^{5}IM2'_{R310L}$ is set to zero.