A Density Functional Theory Study on the Catalytic Mechanism of Hydroxycinnamoyl-CoA Hydratase-Lyase

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Hydroxycinnamoyl-CoA hydratase-lyase (HCHL), a particular member of the crotonase superfamily, catalyzes the bioconversion of feruloyl-CoA to the important flavor and fragrance compound vanillin. In this article, the catalytic mechanism of HCHL has been studied by using hybrid density functional theory method with simplified models. The calculated results reveal that the mechanism involves the hydration of the C═C double bond of feruloyl-CoA and thence the cleavage of C—C single bond of β-hydroxythioester. The hydration step is a typical concerted process, whereas C—C bond cleavage follows a concerted but asynchronous mechanism. The calculated energy barrier of hydration reaction is only slightly lower than that of cleavage process, implying both of two processes are rate limiting. By using three substrate analogs, the substrate specificity of HCHL was further examined. It is found that the *p*-hydroxyl group of aromatic ring is necessary for the catalytic reaction. © 2013 Wiley Periodicals, Inc.

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Introduction

In recent years, numerous mechanistic and structural studies have focused on the crotonase superfamily (CS) because it catalyzes a very wide range of reactions, such as alkene hydration/isomerization,[1,2] aryl-halide dehalogenation,[3] (de)carboxylation,[4] CoA ester and peptide hydrolysis,[5] fragmentation of β-diketone,[6] C—C bond formation, cleavage and oxidation reactions,[7,8] and each of them plays significant role in the biocatalysis and industrial application.

One interesting CS enzyme is hydroxycinnamoyl-CoA hydratase-lyase (HCHL), which catalyzes the biotransformation of feruloyl-CoA to acetyl-CoA and vanillin, as shown in Scheme 1.[10–15] The product vanillin (4-hydroxy-3-methoxybenzaldehyde) is a world's principal flavoring compound,[16] used extensively in the food industry in ice cream, chocolate, and confectionery products. The substrate ferulic acid is a biologically important and abundant molecule, and functions in the cross-linking of plant cell walls.[17] The microbial degradation of phenolic compounds from plant residues is also of environmental and economic importance. The transformation of ferulic acid to vanillin has been thought to be catalyzed by two enzymes (see Scheme 1). First, feruloyl-CoA is formed by the action of adenosine triphosphate (ATP)-dependent 4-hydroxycinnamate-CoA ligase–synthetase, and then the feruloyl-CoA converts to vanillin catalyzed by HCHL through two successive steps involving the first hydration of double bond of feruloyl-CoA and sequent cleavage of β-hydroxy thioester by retro-aldol reaction.

To illuminate the reaction mechanism and substrate specificity, the crystal structure of native HCHL apoenzyme from the gene expressed in *E. coli* has been determined to a resolution of 1.8 Å [Protein Data Bank (PDB) code: 2J5I].[9] Similar with other members of CS, HCHL is a hexamer and its structure superimposed well with that of enoyl-CoA hydratase (ECH), with a root-mean-square deviation of 1.64 Å for 215 matched residues.[7] As both of them can catalyze the conjugate addition of water to α, β-unsaturated thioesters, their catalytic residues were compared. In ECH, two glutamate residues (Glu144 and Glu164) were observed to bind the catalytic water molecule for the hydration reaction of C═C double bonds, whereas in HCHL only one residue (Glu164) was conserved, the residue Glu144 in ECH was replaced by a serine residue (Ser123),[7] which means only the conserved Glu164 is essential for the hydration. Kinetics analysis and mutagenesis experiments of HCHL also proved that Ser123 played no major role in the hydration reaction, and Glu143 was absolutely essential for the hydration of C═C double bond of feruloyl-CoA.[7,9] Similar to other members of CS, a conserved "oxyanion hole" was also formed by the backbone amide nitrogens of Met70 and Gly120 (Gly141 and Ala98 in ECH) to orientate the carbonyl of acyl-CoA thioester and to stabilize the enolate intermediates in HCHL.[7,18,19] The HCHL also shows substrate specificity, which mostly attributes to the strong hydrogen bonds between the phenolichydroxyl group of feruloyl-CoA and the phenolichydroxyl group of Tyr75 and Tyr239 from a neighboring subunit, that is, the *p*-hydroxyl group of aromatic ring of substrate was absolutely essential for ligand recognition and enzymatic activity in HCHL.[7,20]

In summary, several experiments have been conducted in understanding the catalytic mechanism of HCHL.[7,9,21] The binding modes of HCHL with substrates, the different functions of pocket residues, and the catalytic mechanism have been proposed, as shown in Scheme 2. The residues Tyr75 and Tyr239 induce the deprotonation of the phenolic hydroxyl of

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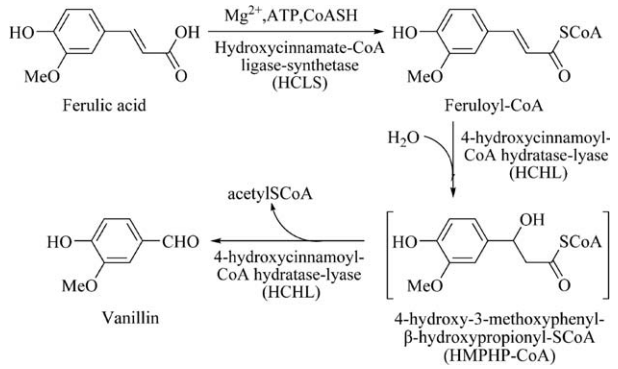


Scheme 1. Biotransformation of ferulic acid to vanillin by *P. fluorescens* AN103.[7,9]

feruloyl-CoA, generating a quinone-methide-enolate intermediate (QME). The catalytic water molecule formed a hydrogen bond network with the side chain of Glu143 and the peptidic NH group of Gly151, making the whole enzymatic reaction possible.[9] Residue Glu143 activates the water molecule to hydrolyze the QME, and subsequent Glu143 protonates the C=C double bond, leading to the formation of intermediate 4-hydroxy-3-methoxyphenyl-β-hydroxypropionyl-SCoA (HMPHP-CoA). Finally, the Glu143 residue acts as the acid/base to catalyze the cleavage of β-hydroxy thioester.

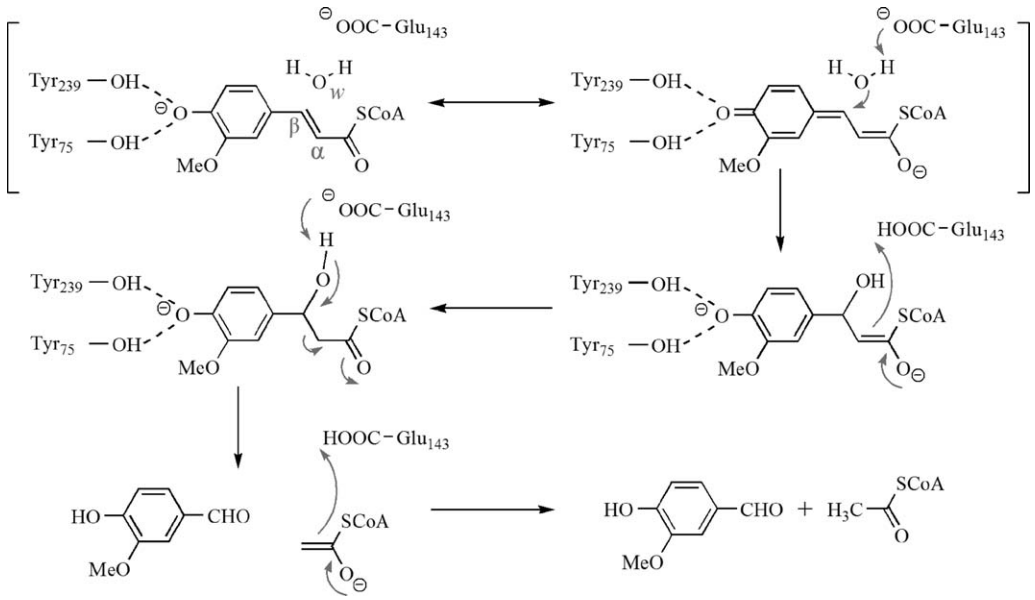
Although a rough outline of catalytic mechanism of HCHL has been drawn, open questions still remain. The rate-limiting step, the energetics of the whole degradation process, and how the substrates influence the catalytic reaction are still unclear. Furthermore, the mechanistic studies on the CS catalytic reactions using theoretical approaches are still very limited, and some valuable information about the HCHL-catalyzed transformation can not be obtained through experimental approaches alone. Therefore, theoretical studies on the catalytic mechanism of HCHL at the atomistic level are required.

In the present article, a hybrid density functional theory (DFT) method,[22–27] which has been testified to be successful in studying enzyme active sites and reaction mechanisms,[28–32] was used to investigate the catalytic mechanism of HCHL. The detailed energetic profile of the overall reaction, and the structures of all intermediates and transition states along the reaction pathway were presented.

Computational Details

As the crystal structure of the binary complex of HCHL and substrate (feruloyl-CoA) is still not available, the computational model used in this work was derived from the X-ray crystal structure of HCHL in complex with vanillin and acetyl-CoA (PDB code: 2VSS), a ternary complex that the catalytic reaction has been finished, as shown in Figure 1. By comparing the structures of apoenzyme of HCHL and the above ternary complex, we found that the positions of pocket residues in the two crystal structures are well superimposed except a minor change of residues Tyr239 and Tyr75. Therefore, according to the principle of microreversibility, the ternary complex may be a reasonable model to study the catalytic mechanism of HCHL. To improve computational efficiency, our model only contains the vanillin, acetyl-CoA, and some key residues (Glu143, Gly151, Tyr239, Tyr75, Met70, and Gly120), in which acetyl-CoA and all residues are truncated, as shown in Figure 2. It should be noted that, in our calculation, the optimized structure (labeled as P) shown in Figure 2 was used as the starting structure to explore the reaction mechanism. In this model, the truncated atoms were fixed to their crystallographic positions to avoid unrealistic movements of the groups, and the fixed atoms were marked with asterisks in Figure 2.

All calculations were performed by using the DFT method with B3LYP function implemented in Gaussian03 program package.[33] Geometry optimizations were performed using



Scheme 2. Proposed mechanism for HCHL-catalyzed transformation of feruloyl-CoA.[7,21]

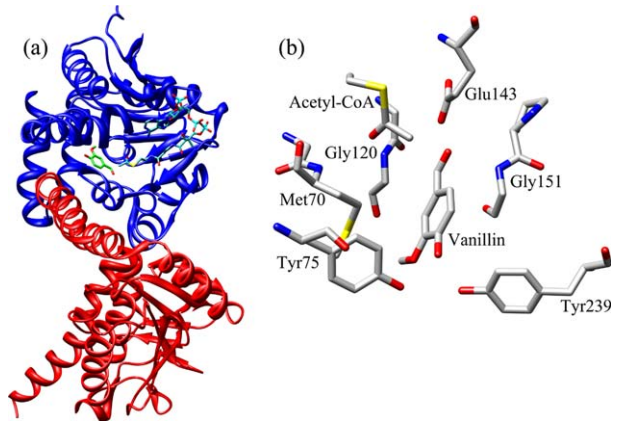


Figure 1. (a) The X-ray crystal of HCHL (PDB code: 2VSS, Chain D and E); (b) Structure of the active site model taken from the crystal. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the 6–31G(d,p) basis set.[28–30] On the basis of these optimized geometries, single-point calculations with the larger basis set 6–311++G(2d,2p) were performed to obtain more accurate energies. Solvent effects were calculated by single-point calculations based on the optimized geometries in the vacuum, using the polarizable continuum model (PCM) at the B3LYP/6–311++G(2d,2p) level.[34,35] In this model, a cavity containing the solute around the system is surrounded by a constant dielectric medium, and two dielectric constants, $\varepsilon = 4$ and $\varepsilon = 80$,[28–30] which have been widely used to simulate the protein and aqueous solvent environments, respectively, were used in the PCM calculations. Frequency calculations were performed at 6–31G(d,p) theory level on all optimized geometries to obtain zero-point vibrational energies and confirm the nature of the stationary points, with no imaginary frequency for local minimum and only one imaginary frequency for saddle points. The freezing procedure used in all calculations usually lead to a few small imaginary frequencies, typically in the order of 10 cm^{-1}. These frequencies do not contribute significantly to the zero-point energies and can thus be ignored. All the transition states have been confirmed by intrinsic reaction coordinate calculations.[36,37]

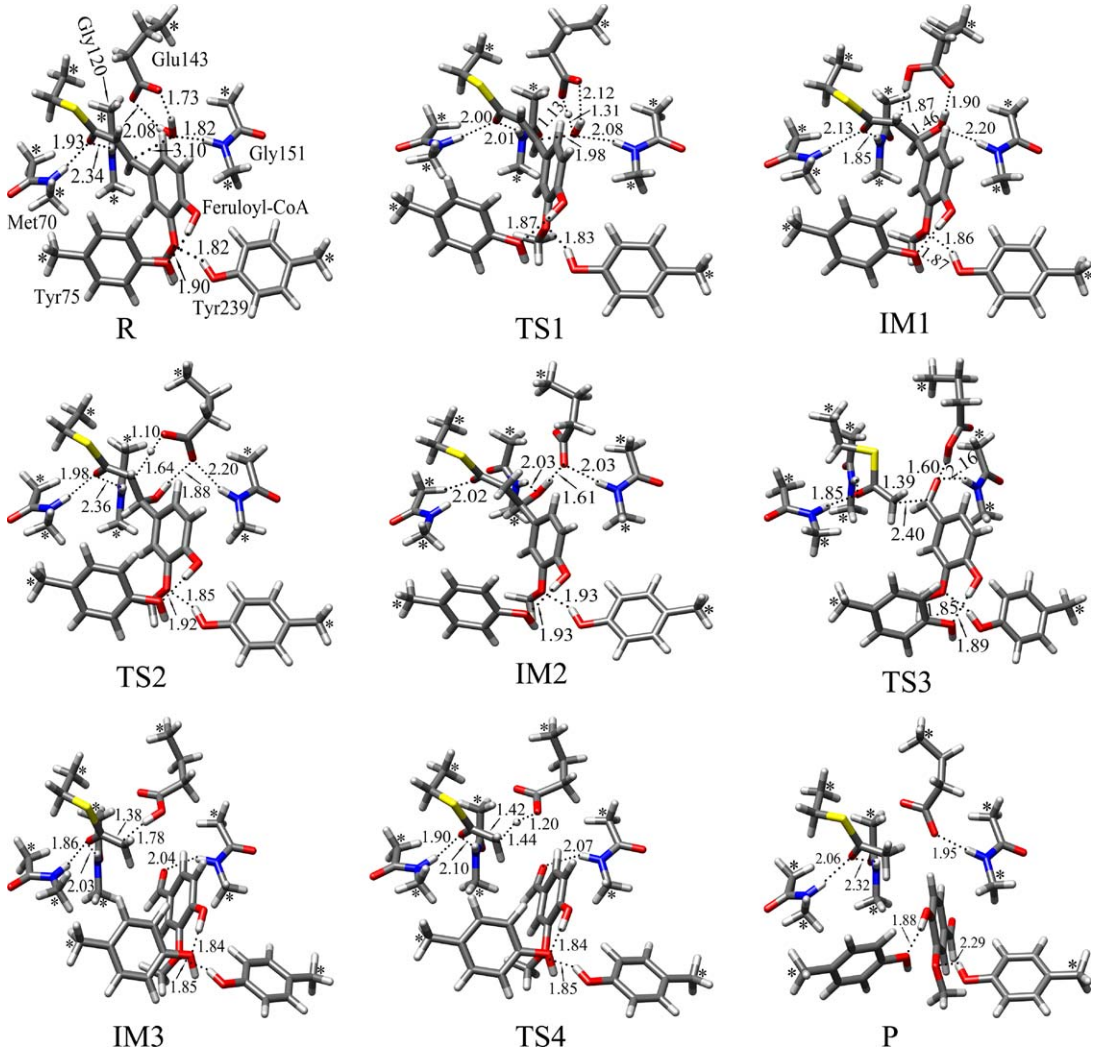


Figure 2. Optimized structures of reactant (*R*), transition states (TS1, TS2, TS3, TS4), intermediates (IM1, IM2, IM3), and product (*P*). Distances are given in Å. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

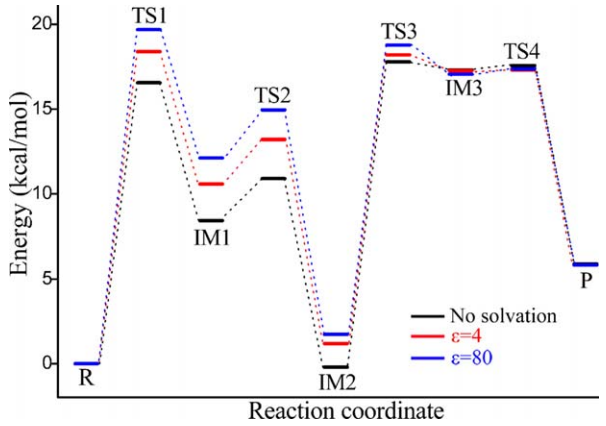


Figure 3. Energy profiles of the HCHL catalytic process. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Results and Discussions

HCHL has been proposed to combine two activities in one net reaction, that is, the hydration of C=C double bond of feruloyl-CoA and thence the cleavage of C—C single bond of the β-hydroxythioester.[7,9,21] Although all the transition states and intermediates were derived from the structure of P, as shown in Figure 3, to conveniently elucidate the reaction mechanism, we still discuss the reaction following the forward order, that is, the hydration reaction from the reactant (R) will occur first, then the cleavage reaction of C—C bond follows.

Hydration of carbon–carbon double bond of feruloyl-CoA

The hydration of C=C double bond of feruloyl-CoA in HCHL keeps a certain similarity with the ECH-catalyzed reaction.[1,38,39] The optimized structure of reactant, all transition states, and intermediates are shown in Figure 2. The Cartesian coordinates and the values of negative frequencies of all stationary points are shown in Supporting Information, Tables S1 and S2, respectively. The energy profiles are shown in Figure 3. In reactant (R), the residues Tyr75 and Tyr239 form strong hydrogen bonding interactions with the phenolichydroxyl and methoxyl of feruloyl-CoA with distances of 1.90 and 1.82 Å, respectively. The carbonyl group of feruloyl-CoA forms two hydrogen bonds with the backbone NH groups of Met70 and Gly120 (1.93 and 2.34 Å length, respectively). In addition, a hydrogen bond network is formed between the catalytic water molecule and the carboxyl group of Glu143 and the backbone amine NH of Gly151, which is in well agreement with the experimental observations.[9] The oxygen (O_w) of water molecule is in suitable orientation for ideal nucleophilic attack on the benzylic C atom (C_β) of feruloyl-CoA with a distance of about 3.10 Å, which is almost the same (~3.30 Å) as mentioned by Leonard.[9] Thus, the substrate feruloyl-CoA locates in the center of active site and the catalytic water molecule is situated in the right position to hydrate the C=C double bond.

Figure 3 shows that the hydration of carbon–carbon double bond of feruloyl-CoA contains two elementary steps, and IM2 is the final product of hydration. In TS1, the Glu143 acts as a general base to capture a proton of water molecule, and the resulting hydroxyl group attacks the C_β atom of feruloyl-CoA. The distance between C_β and O_w changes to 1.98 Å from 3.10 Å, and the distance between the hydrogen atom of water molecule and the carboxyl oxygen atom of Glu143 changes from 2.08 Å to 1.13 Å. Thus, the hydration of C_α—C_β double bond follows a concerted mechanism, leading to the formation of a negatively charged enolate intermediate IM1, which is stabilized by an "oxyanion hole" formed by the backbone amide nitrogens of Met70 and Gly120. In IM1, the C_β—O_w bond distance changes to 1.46 Å from 1.98 Å. The calculated relative energies are summarized in Table 1. The energy barrier to generate IM1 is 16.54 kcal/mol, which is thermodynamically practicable in microorganisms. The IM1 is higher than R in energy by 8.45 kcal/mol.

TS2 corresponds to a transition state of proton transfer from Glu143 to the C_α of IM1 with imaginary frequency of 125.32i cm^{-1}. The calculated energy barrier is only 2.45 kcal/mol relative to IM1, indicating the proton transfer is very facile. The proton transfer leads to a very stable intermediate IM2, which is also called as HMPHP-CoA, as shown in Scheme 1. The IM2 is calculated to be 0.21 kcal/mol lower than reactant, and HMPHP-CoA has been experimentally detected by Mitra et al.[20]

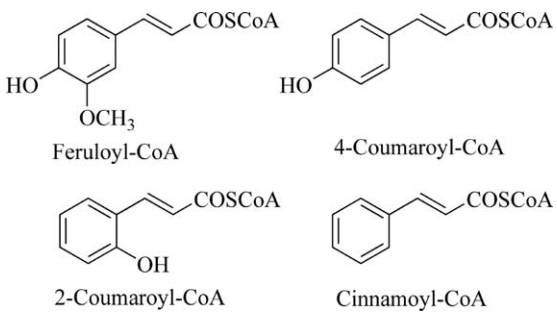
To approximately estimate the effects of protein electrostatic and aqueous solution surroundings on the energy barriers, the single point energies were further calculated by using PCM at the level of 6–311++G(2d,2p) with dielectric constants of 4 and 80, respectively. As shown in Table 1, to generate IM1, the calculated energy barriers on PCM model are slightly increased to 18.39 ($\varepsilon = 4$) and 19.69 kcal/mol ($\varepsilon = 80$), implying that the enzyme and aqueous solution environments have great influences on the energetics of hydration reaction.

Cleavage of carbon–carbon bond of β-hydroxythioester

As shown in Scheme 2 and Figure 3, the whole cleavage of carbon–carbon bond of β-hydroxythioester involves two steps. In the first step, IM3 is formed by the cleavage of carbon–

Table 1. Summary of the calculated relative energies (kcal/mol) for the catalytic reaction.

	R	TS1	IM1	TS2	IM2	TS3	IM3	TS4	P
No solvation	0.00	16.54	8.45	10.90	−0.21	17.78	17.30	17.56	5.89
No solvation with BSSE correction	0.00	17.61	8.23	10.65	−0.99	17.40	17.15	17.21	5.66
$\varepsilon = 4$	0.00	18.39	10.57	13.21	1.19	18.20	17.27	17.30	5.82
$\varepsilon = 80$	0.00	19.69	12.11	14.94	1.74	18.77	17.06	17.35	5.83



Scheme 3. Substrate of the HCHL and its three different analogs.

carbon bond. Then, a proton transfers from Glu143 to CH_2═C(OH) —SCoA enolate intermediate to complete the catalytic reaction.

TS3 corresponds to the transition state of the cleavage of carbon–carbon bond with imaginary frequency of 86.94i cm^{-1}. In TS3, the C_α—C_β distance increases from 1.55 to 2.40 Å. The proton of β-hydroxyl group of HMPHP-CoA has been transferred to the oxygen atom of carboxyl of Glu143. By comparing the geometries of IM2 and TS3, we note that the proton transfer and cleavage of C_α—C_β single bond is a concerted but asynchronous process. The calculated energy barrier of this step is 17.99 kcal/mol. In IM3, the fragrance compound vanillin and an unstable enolate intermediate are generated. To complete the catalytic cycle, the enolate intermediate extracts a proton from the protonated Glu143 residue to yield acetyl-CoA. The energy barrier of proton transfer is calculated to be 0.26 kcal/mol, meaning the proton transfer is a very facile process.

As shown in Figure 3 and Table 1, PCM solvation effects show minor influence on the energies of the cleavage of carbon–carbon bond of β-hydroxythioester.

From the energy point of view, the calculated energy barrier of hydration of C═C double bond of feruloyl-CoA is only slightly lower than that of the cleavage of C—C bond of HMPHP-CoA (16.54 vs. 17.99 kcal/mol), implying both of two processes are rate-limiting.

To eliminate the basis set superposition error (BSSE), the single-point energy calculations with the basis set of 6–311++G(2d,2p) including BSSE correction were also performed. The detailed calculation results are shown in Table 1 and Supporting Information, Table S4. We can see that values of BSSE are minor and can be ignored.

As the whole reaction system seems to be affected by dispersion, we have also calculated zero-point energies and single-point energies by using M06 method, and the calculated energies are shown in Supporting Information, Table S5. By comparing the relative energies calculated using B3LYP and M06 methods, we notice that the dispersion effect has a certain impact on the relative energies (especially IM2, TS3, and P). However, the dispersion effect does not hinder the feasibility of the proposed reaction mechanism of HCHL.

Substrate specificity of HCHL

Experimental studies about the substrate specificity of HCHL indicated that the *p*-hydroxyl group of aromatic substrate is necessary for HCHL-catalyzed reaction. To examine the substrate specificity of HCHL, three feruloyl-CoA analogs, including 4-coumaroyl-CoA, cinnamoyl-CoA and 2-coumaroyl-CoA (shown in Scheme 3), were used to calculate the key steps of the catalytic reaction. The calculated energies of three substrate analogs are shown in Supporting Information, Table S6.

As shown in Figure 4a, for 4-coumaroyl-CoA, the calculated energy barriers of hydration of C═C double bond and cleavage reaction of C—C single bond are 15.52 and 16.49 kcal/mol, respectively, slightly lower than those of feruloyl-CoA (16.54 and 17.99 kcal/mol, respectively). By examining the structures shown in Figure 4a, we can see that the *p*-hydroxyl forms strong hydrogen bonds with Tyr239 and Tyr75, which effectively facilitates the catalytic reaction. However, for 2-coumaroyl-CoA and caffeoyl-CoA (Figs. 4b and 4c), all the energy barriers of key steps are increased significantly due the absence of *p*-hydroxyl of substrates, which agree well with the experimental observations that both of the substrates are unresponsive to HCHL.[20]

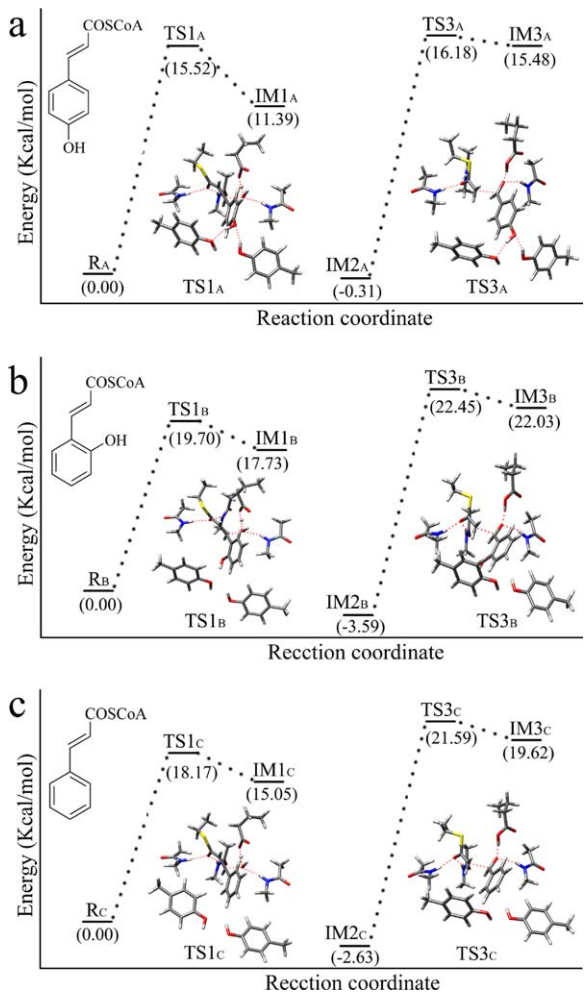


Figure 4. Energy profiles of the hydration and cleavage reaction of three different substrate analogs: (a) 4-coumaroyl-CoA; (b) 2-coumaroyl-CoA; (c) cinnamoyl-CoA. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Conclusions

In the present study, we have investigated the catalytic mechanism of HCHL by using DFT method. The calculated results reveal that the whole reaction process contains four elementary steps, including one concerted hydration reaction of C═C double bond, one concerted but asynchronous cleavage reaction of C—C single bond, and two proton transfer steps. Glu143 is absolutely essential to act as the only acid/base to participate the two proton transfer processes. The calculated energy barrier of hydration of C═C double bond is only slightly lower than the cleavage of C—C single bond, meaning both the hydration and cleavage may be rate-limiting.

The calculations on three feruloyl-CoA analogs show that the *p*-hydroxyl group of aromatic substrate is necessary for catalytic reaction. Residues Tyr75 and Tyr239 form strong hydrogen bonds with the *p*-hydroxyl groups of substrates. Without the *p*-hydroxyl groups, the energy barriers of the key steps will increase significantly.

Overall, our calculation results provide strong support to the proposed mechanism of HCHL-catalyzed biotransformation of feruloyl-CoA, which may be used as a good reference for the study of other members of CS.

Keywords: hydroxycinnamoyl-CoA hydratase-lyase · density functional theory method · catalytic mechanism · substrate specificity

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Additional Supporting Information may be found in the online version of this article.

[1] B. J. Bahnson, V. E. Anderson, G. A. Petsko, *Biochemistry* **2002**, *41*, 2621.
[2] A. M. Mursula, D. M. F. van Aalten, J. K. Hiltunen, R. K. Wierenga, *J. Mol. Biol.* **2001**, *309*, 845.
[3] M. M. Benning, K. L. Taylor, R. Q. Liu, G. Yang, H. Xiang, G. Wesenberg, D. Dunaway-Mariano, H. M. Holden, *Biochemistry* **1996**, *35*, 8103.
[4] M. M. Benning, T. Haller, J. A. Gerlt, H. M. Holden, *Biochemistry* **2000**, *39*, 4630.
[5] B. J. Wong, J. A. Gerlt, *J. Am. Chem. Soc.* **2003**, *125*, 12076.
[6] G. Grogan, *Biochem. J.* **2005**, *388*, 721.
[7] J. P. Bennett, L. Bertin, B. Moulton, I. J. S. Fairlamb, A. M. Brzozowski, N. J. Walton, G. Grogan, *Biochem. J.* **2008**, *414*, 281.
[8] E. D. Eberhard, J. A. Gerlt, *J. Am. Chem. Soc.* **2004**, *126*, 7188.
[9] P. M. Leonard, A. M. Brzozowski, A. Lebedev, C. M. Marshall, D. J. Smith, C. S. Verma, N. J. Waltonc, G. Grogan, *Acta Crystallogr.* **2006**, *D62*, 1494.
[10] A. Narbad, M. J. Gasson, *Microbiology* **1998**, *144*, 1397.
[11] N. J. Walton, A. Narbad, C. B. Faulds, G. Williamson, *Curr. Opin. Biotechnol.* **2000**, *11*, 490.
[12] M. J. Gasson, Y. Kitamura, W. R. McLauchlan, A. Narbad, A. J. Parr, E. L. H. Parsons, J. Payne, M. J. C. Rhodes, N. J. Waltoni, *J. Biol. Chem.* **1998**, *273*, 4163.
[13] J. Overhage, H. Priefert, A. Steinbüchel, *Appl. Environ. Microbiol.* **1999**, *65*, 4837.
[14] R. Plaggenborg, J. Overhage, A. Steinbüchel, H. Priefert, *Appl. Microbiol. Biotechnol.* **2003**, *61*, 528.
[15] A. Muheim, K. Lerch, *Appl. Microbiol. Biotechnol.* **1999**, *51*, 456.
[16] N. J. Walton, M. J. Mayer, A. Narbad, *Phytochemistry* **2003**, *63*, 505.
[17] S. Y. Ou, K. C. Kwok, *J. Sci. Food Agric.* **2004**, *84*, 1261.
[18] R. B. Hamed, E. T. Batchelar, I. J. Clifton, C. J. Schofield, *Cell. Mol. Life Sci.* **2008**, *65*, 2507.
[19] H. M. Holden, M. M. Benning, T. Haller, J. A. Gerlt, *Acc. Chem. Res.* **2001**, *34*, 145.
[20] A. Mitra, Y. Kitamura, M. J. Gasson, A. Narbad, A. J. Parr, J. Payne, M. J. C. Rhodes, C. Sewter, N. J. Walton, *Arch. Biochem. Biophys.* **1999**, *365*, 10.
[21] J. F. Jin, U. Hanefeld, *Chem. Commun.* **2011**, *47*, 2502.
[22] A. D. Becke, *Phys. Rev. A* **1988**, *38*, 3098.
[23] A. D. Becke, *J. Chem. Phys.* **1992**, *96*, 2155.
[24] A. D. Becke, *J. Chem. Phys.* **1992**, *97*, 9173.
[25] A. D. Becke, *J. Chem. Phys.* **1993**, *98*, 5648.
[26] C. Lee, W. Yang, R. G. Parr, *Phys. Rev. B* **1998**, *37*, 785.
[27] B. Miehlich, A. Savin, H. Stoll, H. Preuss, *Chem. Phys. Lett.* **1989**, *157*, 200.
[28] (a) P. Georgieva, F. Himo, *J. Comput. Chem.* **2010**, *31*, 1707; (b) R. Z. Liao, J. G. Yu, F. Himo, *Inorg. Chem.* **2009**, *48*, 1442; (c) P. Velichkova, F. Himo, *J. Phys. Chem. B* **2005**, *109*, 8216; (d) S. L. Chen, W. H. Fang, F. Himo, *J. Phys. Chem. B* **2007**, *111*, 1253; (e) P. Georgieva, Q. Wu, M. J. McLeish, F. Himo, *Biochim. Biophys. Acta* **2009**, *1794*, 1831.
[29] L. Yang, R. Z. Liao, J. G. Yu, R. Z. Liu, *J. Phys. Chem. B* **2009**, *113*, 6505.
[30] M. Feliks, G. M. Ullmann, *J. Phys. Chem. B* **2012**, *116*, 7076.
[31] L. J. Zhao, X. Y. Ma, R. G. Zhong, *Int. J. Quantum Chem.* **2013**, *113*, 1299.
[32] X. Li, Q. C. Zheng, H. X. Zhang, *Int. J. Quantum Chem.* **2012**, *112*, 619.
[33] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, J. A. Pople, Gaussian 03 (Revision D.01), Gaussian, Inc., Wallingford CT, **2004**.
[34] V. Barone, M. Cossi, J. Tomasi, *J. Comput. Chem.* **1998**, *19*, 404.
[35] J. Tomasi, M. Persico, *Chem. Rev.* **1994**, *94*, 2027.
[36] C. Gonzalez, H. B. Schlegel, *J. Chem. Phys.* **1989**, *90*, 2154.
[37] C. Gonzalez, H. B. Schlegel, *J. Phys. Chem.* **1990**, *94*, 5523.
[38] G. Agnihotri, H. W. Liu, *Bioorg. Med. Chem.* **2003**, *11*, 9.
[39] C. K. Engel, M. Mathieu, J. P. Zeelen, J. K. Hiltunen, R. K. Wierenga, *EMBO. J.* **1996**, *15*, 5135.

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