乳胶清除蛋白Lcp_{K30}催化氧气裂解聚顺-1,4异戊二烯机理

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摘要 乳胶清除蛋白Lcp_{K30}是一种内型双加氧酶, 其通过活化分子氧催化裂解化学惰性的聚顺-1,4异戊二烯, 进而生成醛和酮。实验发现活性区域的谷氨酸148对活性有重大影响, 然而其作用机制和整个反应机理仍然存在争议。本研究使用量子力学/分子力学组合方法研究了乳胶清除蛋白催化分子氧氧化裂解天然橡胶模型化合物的反应机理。计算结果显示谷氨酸148在反应过程中为质子化态, 并与铁(III)-超氧物种形成稳定氢键, 因此其作用可能与调节铁(III)-超氧物种与底物的位置有关。可能反应机理为: (1) 铁(III)-超氧物种的远端氧进攻底物碳碳双键, 形成过氧烃自由基中间体; (2) 近端氧回弹到碳自由基, 形成过氧化物中间体和铁(II)中心; (3) 铁(II)调节的过氧化物中间体O-O键还原裂解, 生成邻二醇自由基负离子中间体; (4) 邻二醇自由基负离子C-C键断裂, 生成产物醛和酮。此外, 计算发现, 过氧化物中间体O-O键直接断裂反应能垒超过160 kJ/mol, 表明铁(II)的催化对于过氧化物中间体的后续转化至关重要。因此, 本研究结果不仅加深对于Lcp_{K30}反应机理的理解, 而且为Lcp酶降解合成橡胶材料的设计和工程化提供有用信息。

关键词 乳胶清除蛋白, 催化, 量子力学/分子力学组合方法, 反应机理, 橡胶降解, 血红素酶

天然橡胶和合成橡胶(聚顺-1,4异戊二烯)材料已经广泛应用于我们的日常生活和工业生产中。然而, 其每年的高生产量和高消耗量给降解废弃产品带来了巨大挑战。目前废弃橡胶的主要处理方法为燃烧和集中堆积, 这些处理方法对生态环境危害很大。因此, 开发回收橡胶的方法十分必要^[1]。生物降解法因其环境友好性和可持续性, 是一种很好的潜在方法, 催化裂解惰性橡胶的酶已受到广泛关注。

目前, 已经报道了两种细胞外的橡胶清除酶。其中一种是橡胶氧化酶(rubber oxygenase, RoxA)。RoxA首次从黄单胞菌*Xanthomonas* sp. 35Y中获得^[2,3], 随后也在其他革兰氏阴性的橡胶降解细菌中获得^[4]。RoxA是

一种双血红素的c型细胞色素, 催化裂解聚顺-1,4异戊二烯得到15个碳的主要产物^[5,6]。另一种橡胶氧化酶是乳胶清除蛋白(latex clearing protein, Lcp)。Lcp首次由链球菌*Streptomyces* sp. K30(Lcp_{K30})获得^[7], 后来也在戈登氏菌^[8~10]、紫红红球菌^[11]和其他链球菌^[12]中得到。近来, 实验上得到了3种乳胶清除蛋白即Lcp_{VH2}、Lcp_{K30}和Lcp_{Rt}的纯化和生化表征结果^[11,13~16]。与RoxA不同, Lcp是一种b型细胞色素^[11,15], 催化裂解聚顺-1,4异戊二烯得到醛和酮^[14,17]。

文献^[18]报道了两种Lcp_{K30}的晶体结构, 如图1(a), (b)所示, 分别为开放构型(PDB编号: 5O1L)和关闭构型(PDB编号: 5O1M)。在开放构型Lcp_{K30}晶体结构中, 一

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图 1 (网络版彩色)Lcp_{K30}两种构型的晶体结构和两种提出的反应路径^[18]. (a) Lcp_{K30}开放构型的晶体结构(PDB编号: 5O1L); (b) Lcp_{K30}关闭构型的晶体结构(PDB编号: 5O1M); (c) Lcp_{K30}催化裂解聚顺-1,4异戊二烯的可能反应机理
Figure 1 (Color online) Two castral structures and two proposed possible reaction paths^[18]. (a) Crystal structure of open state Lcp_{K30} (PDB ID: 5O1L); (b) crystal structure of closed state Lcp_{K30} (PDB ID: 5O1M); (c) proposed mechanism for Lcp_{K30}-catalyzed cleavage of poly(*cis*-1,4-isoprene)

分子缓冲剂咪唑配位到铁中心;而在关闭构型中,与铁中心配位的是赖氨酸,而仅有开放构型对底物有活性^[18].

一般认为血红素双加氧酶的反应中,分子氧的两个氧原子都键合到底物^[19].文献^[18]提出Lcp_{K30}催化裂解聚顺-1,4异戊二烯有两种可能的反应路径,如图1(c)所示.两种反应路径都提出,谷氨酸(E148)作碱提取烯丙基的质子引发反应.后续反应为氧原子进攻底物的碳碳双键.在路径I中,氧合血红素的两个氧原子分别进攻碳碳双键的两个碳原子,形成过氧烃中间体;在路径II中,分子氧的远端氧加成到碳碳双键,再由O-O键断裂、C-O键成键,形成环氧化物中间体.

与Lcp_{K30}相似的还有两种血红素双加氧酶,即吡啶胺2,3-双加氧酶(IDO)和色氨酸双加氧酶(TDO)^[20,21].近来,血红素-色氨酸模型的密度泛函理论研究^[22]和实验晶体结构测量^[23]证明碱驱动的反应机理是不合理的.因此,Lcp_{K30}催化反应中谷氨酸148作为碱驱动反应也是值得怀疑的.Zhang和Liu^[24]的QM/MM组合方法研究发现,谷氨酸148不能作为碱提取烯丙基的质子而驱动反应;整个反应经过两次C-O键成键形成过氧化物中

间体,再发生C-C键和O-O键协同断裂形成产物.然而,结果中并没有提出谷氨酸148的作用机制,提出的反应机理中过氧中间体过氧键直接断裂(能垒仅约84 kJ/mol),相对于过氧烃O-O键键能的150 kJ/mol^[25]过于容易.此外,一些非血红素双加氧酶如类胡萝卜素氧化酶(ACO)^[26]和芪类裂解氧化酶(SCO)^[27]的研究也为深入理解Lcp_{K30}反应机理提供了参考.

总体上,尽管有研究提出Lcp_{K30}的可能反应机理,然而依然不够完善,谷氨酸148的作用机制和反应机理细节不够明确.研究Lcp_{K30}催化体系可以为理解Lcp催化反应机制和相应高效仿生催化剂提供理论基础.本研究采用量子力学/分子力学(quantum mechanics/molecular mechanics, QM/MM)组合方法研究了Lcp_{K30}催化体系的反应机理,谷氨酸148在质子态的作用机制和Fe中心在过氧化物裂解中的作用.

1 计算方法

(i) 体系设置.本研究的计算模型基于Lcp_{K30}的开放构型(PDB编号: 5O1L),由于其中两条链是相同的,故只取其中一条链(A链),移除了结晶缓冲剂咪唑,手

动添加氧气配位到铁中心. 底物聚顺-1,4异戊二烯在模型中由9个重复单元的短链代表. Chimera^[28]的Auto Dock Vina^[29]工具用以将底物对接到Lcp_{K30}活性区域. 可滴定氨基酸的质子态基于PROPKA^[30]计算的pK_a值, 并通过可视化软件VMD检查附近的氢键网络确定. 谷氨酸148(pK_a=9.04和天冬氨酸174(pK_a=7.41)设定为质子化态, 其余谷氨酸和天冬氨酸为脱质子化态; 组氨酸93、203和259质子化在氮ε位; 组氨酸156、238和268质子化在氮δ位; 组氨酸204同时质子化两个氮原子. 使用AmberTools18^[31]中的MCPB.py工具^[32,33]参数化血红素的力场; 蛋白质氨基酸残基力场参数由Amberff14SB力场^[34]获得; 底物的力场参数由Amber的GAFF力场^[35]结合HF/6-31G*水平的RESP方法^[36]获得. 底物缺失的参数由AmberTools18的parmchk功能补充. 在蛋白质表面添加钠离子以平衡体系的电荷. 最后, 将整个体系溶解在超过蛋白质表面18 Å的长方体TIP3P水箱中.

(ii) 分子动力学模拟. 经过上述体系设置, 首先使用最速降和共轭梯度结合法将整个体系充分最小化; 然后, 整个体系在弱的限制下, 从10~300 K进行温和和经典退火50 ps. 为确保加热动力学后密度一致, 在300 K和1 atm下进行了常温常压(NPT)系综下的1 ns密度平衡. 再移除蛋白质的所有限制并在NPT系综法下进行3 ns平衡. 最后, 在NPT系综法下对整个体系进行了50 ns的分子动力学模拟. 所有的分子动力学模拟都在Amber 18程序包内进行^[31].

(iii) QM/MM计算. 分子动力学模拟显示蛋白骨架原子的均方根偏差(RMSD)在2 ns之后达到平衡, 我们选取分子动力学模拟(molecular dynamics, MD)平衡后的代表性快照用于后续的QM/MM. QM/MM由Chemshell^[37,38]完成, Turbomole^[39]进行QM部分计算, DL_POLY^[40]进行MM部分计算. 酶环境对QM区域的极化作用由静电嵌入方法^[41]模拟. QM/MM边界由氢连接原子和电荷转移模型^[38]处理. QM/MM计算中, QM部分使用杂化泛函UB3LYP^[36,42,43]和两个水平的基组计算, 几何结构优化时使用的基组为def2-SVP^[44], 能量校正使用的基组为def2-TZVP^[44]. 所有讨论的内容均经过能量校正和色散校正^[45,46]. QM部分包括两个重复单元代表的底物、不含侧链羧基的血红素、分子氧、组氨酸198的侧链和谷氨酸148的侧链. MM区域为QM区域0.8 nm内残基. 所有的过渡态都通过柔性势能扫描后, 由最高点优化得到.

2 结果与讨论

2.1 酶-底物络合物结构

与非血红素铁双加氧酶^[27,47]类似, Fe(II)与三重态分子氧的结合可产生单重态、三重态、五重态和七重态的Fe(III)-O₂⁻结构. 根据我们的计算(图2), 开壳层单重态为基态, 与文献^[48]报道的氧-卟啉-吡啶配合物结果相符. 七重态结构中Fe(III)为高自旋, 5个单电子与超氧的1个单电子自旋平行; 五重态有两种结构, 分别记为Q1和Q2, Q1中Fe(III)的3个单电子与超氧的1个单电子自旋平行, Q2中Fe(III)为高自旋, 5个单电子与超氧的1个单电子自旋反平行; 三重态有两种结构, 分别记为T1和T2, T1中Fe(III)为低自旋, 1个单电子与超氧的1个单电子自旋平行, T2中Fe(III)的3个单电子与超氧的1个单电子自旋反平行; 开壳层单重态中Fe(III)为低自旋, 1个单电子与超氧的1个单电子自旋反平行. 相对于开壳层单重态, 七重态、五重态和三重态的T2, 能量超过20 kJ/mol, 本研究不予讨论. 三重态的T1能量与开壳层单重态能量接近, 与文献^[49~51]报道QM/MM计

图2 (网络版彩色)Fe(III)-超氧自由基的6种电子态价电子构型和相对能量

Figure 2 (Color online) Electron configurations of Fe(III)-superoxide at six possible electron states, shown below the relative energies

算人类卟啉胺双加氧酶(hIDO)中基态的开壳层单重态能量只比三重态的低2.5 kJ/mol一致。

如图3所示, 单重态结构与三重态结构类似, 三重态结构中氧合血红素远端氧O1与谷氨酸148形成氢键, 同时与底物碳碳双键两个碳原子的距离分别为 $d(\text{O1}-\text{C1})=0.456\text{ nm}$, $d(\text{O1}-\text{C2})=0.405\text{ nm}$ 。与其他双加氧酶底物结合^[27,48,49]时, 底物和活性区域残基形成的氢键通常不同, 聚顺-1,4异戊二烯并没有与周围残基形成强的作用。O-O键键长为0.129 nm, 氧氧表现为超氧特征, 与双加氧酶中的麦角硫因氧化酶^[47]一致。自旋布居分析显示单重态的O1自旋布居数为-0.58, O2自旋布居数为-0.47, Fe自旋布居数为1.12; 三重态的O1自旋布居数为0.42, O2自旋布居数为0.55, Fe自旋布居数为1.11。

2.2 反应机理

谷氨酸148的 pK_a 值为9.04, 表明谷氨酸148处于质子化态, 排除其作为碱提取底物的质子的可能, 与Zhang和Liu^[24]提出的E148提取底物质子不合理的观点一致。

反应可能有两种可能路径, 如图1(c)所示, 现分别讨论。路径I中, 氧合血红素的远端氧(O1)首先进攻底物

图3 (网络版彩色)三重态³RC和单重态⁵S RC反应络合物的结构, 距离单位为nm

Figure 3 (Color online) QM/MM-optimized structure of the reactant complexes at triplet ³RC and singlet ⁵S RC. Distances are given in nanometer

碳碳双键的碳原子C1, 碳氧第一次成键, 经过过渡态TS1生成血红素键合的过氧化物自由基中间体IC1, 如图4所示。三重态下O1-C1距离由³RC的0.456 nm缩短到³TS1的0.190 nm, 到³IC1的0.147 nm, 如图5所示。

图4 (网络版彩色)QM/MM计算的三重态Fe(III)-O₂裂解聚顺-1,4异戊二烯的势能面(kJ/mol)以及关键中间体结构, 路径I为蓝色, 路径II为红色
Figure 4 (Color online) QM/MM calculated energy profiles (in kJ/mol) for Fe(III)-O₂ mediated oxidative cleavage of poly(*cis*-1,4-isoprene) in the triplet state, shown along with the schematic drawings of key intermediates along the reaction pathways (path I in blue and path II in red)

图5 (网络版彩色)QM/MM优化的三重态路径I中的TS1、IC1、TS2和IC2的QM区域结构,距离单位为nm.为清晰显示,省略了一些不重要的H原子

Figure 5 (Color online) QM/MM optimized structures of TS1, IC1, TS2, and IC2 from the QM region along path I in the triplet state. All distances are given in nanometer. Some unimportant H atoms are omitted for clarity

³IC1中O1–O2键长由³RC的0.129 nm伸长到0.145 nm,表现为过氧特征. 碳氧第一次成键过程中,相对于³RC三重态反应能垒为75.7 kJ/mol,相对于^{OS}RC开壳层单重态反应能垒为87.7 kJ/mol. 随着O1–C1成键, C1与C2的键断裂, C1原子由sp²杂化转换为sp³杂化, C2原子自旋布居数为0.97,以自由基形式保持sp²杂化.

从IC1开始,下一步反应为与Fe配位的O2进攻底物的碳原子C2,碳氧第二次成键,生成过氧烃中间体IC2,反应能垒相对³IC1为51.5 kJ/mol. Fe–O2距离由³IC1的

0.188 nm伸长为³IC2的0.240 nm, 谷氨酸148与O1形成氢键. 电子结构分析发现在³IC2中Fe中心的自旋布居数为2.12, 而O1和O2的自旋布居数趋近于0, 表明Fe–O2均裂, Fe(III)还原为Fe(II), 氧自由基O2与碳自由基C2结合成键. 此外, 由于在IC2中, 两个α电子都分布到Fe中心, 因此开壳层单重态并不存在.

形成过氧烃中间体IC2后, 过氧键和C–C键断裂即可形成产物醛和酮. 文献[24]报道, Lcp_{K30}体系的过氧烃中间体通过过氧键和C–C键直接协同断裂生成产物,

能垒超过83 kJ/mol. 计算发现从 $^3\text{IC2}$ 开始, O—O键和C—C键断裂为分步进行, 首先发生O—O键断裂, 经过过渡态 $^3\text{TS3}$, 生成 $^3\text{IC3}$, 如图4和6所示. $^3\text{IC3}$ 中Fe—O2键长缩短至0.182 nm, 自旋布居分析发现Fe中心自旋布居数为1.02, O1和O2自旋布居数分别为0.69和0.35, 表明O—O键断裂是由Fe中心提供一个电子驱动的, Fe由+2价氧

化为+3价. 该过程反应能垒为33.9 kJ/mol, 反应放热超过62 kJ/mol. 最后, $^3\text{IC3}$ 通过C1—C2和Fe—O2断裂, 生成产物醛和酮, Fe中心由+3价还原为+2价, 反应经过过渡态 $^3\text{TS4}$, 能垒相对于 $^3\text{IC3}$ 为36 kJ/mol, 并放热超过250 kJ/mol.

此外, 采用DFT方法在相同水平计算了气相过氧

图6 (网络版彩色)QM/MM优化的三重态路径I中的TS3、IC3、TS4和PD的QM区域结构, 距离单位为nm. 为清晰显示, 省略了一些不重要的H原子

Figure 6 (Color online) QM/MM optimized structures of TS3, IC3, TS4, and PD from the QM region along the path I in the triplet state. All distances are given in nanometer. Some unimportant H atoms are omitted for clarity

化物O—O键和C—C键协同断裂的反应,如图S1示.结果显示没有外界电子引入,O—O键和C—C键协同断裂反应能垒超过160 kJ/mol,表明Fe在IC2的O—O键断裂过程中起到关键作用,谷氨酸148的固定作用可能有助于Fe和过氧烃的配位和电子传递.

综上所述,经过过氧烃中间体的路径I在能量上是可行的,整个碳碳双键的氧化裂解是高度放热的.

经过环氧烃中间体的路径II如图4和7所示,氧合血

红素的远端氧O1进攻底物碳碳双键的C2,形成³IC1a.自旋布居分析发现,C1的自旋布居数为1.03而O1和O2的自旋布居数分别为0.02和0.13,表明IC1a为过氧化物.这一反应过渡态为³TS1a,反应能垒相对于³RC为82.9 kJ/mol.³IC1a可能形成过氧烃和环氧烃两种中间体.在³IC1a中,与Fe配位的O2与底物碳碳双键的碳原子C1处于反式构型,O2—C1距离为0.377 nm,这一构型不利于形成过氧烃中间体.文献[24]报道形成过氧烃反

图7 (网络版彩色)QM/MM优化的三重态路径II中的TS1a、IC1a、TS2a和IC2a的QM区域结构,距离单位为nm.为清晰显示,省略了一些不重要的H原子

Figure 7 (Color online) QM/MM optimized structures of TS1a, IC1a, TS2a, and IC2a from the QM region along the path II in the triplet state. Distances are given in nanometer. Some unimportant H atoms are omitted for clarity

图8 (网络版彩色)根据QM/MM计算提出的Lcp_{K30}催化裂解聚顺-1,4异戊二烯的反应机理

Figure 8 (Color online) Proposed mechanism of Lcp_{K30} catalyzed oxidative cleavage of poly(*cis*-1,4-isoprene) according to the QM/MM calculations

应能垒超过117 kJ/mol, 故在本研究中不讨论生成过氧烃中间体的反应路径. ³IC1a通过形成O1-C1和断裂O1-O2生成³IC2a, 反应能垒相对于³IC1a为50.1 kJ/mol, 放热超过90 kJ/mol. ³IC2a中的Fe=O配合物与其他P450酶^[52]类似. 环氧烃中间体可通过Fe=O进攻C2, 生成产物, 然而计算发现这一反应能垒超过160 kJ/mol. 故路径II不是可能的反应路径.

3 结论

本研究使用QM/MM组合方法研究了乳胶清除蛋白Lcp_{K30}氧化裂解底物的反应机理. 计算结果表明, 谷氨酸148不能如前人推测作碱提取底物的质子, 而是保持质子化状态在反应中与氧合血红素和过氧中间体形成氢键, 不仅调节氧合血红素和底物的相对位置, 也固定氧化物中间体使其与铁中心配位, 有利于铁的电子传递进而促进O-O键的断裂. 且DFT计算发现过氧烃中间体在无电子引入下, O-O键和C-C键协同断裂反应能垒超过160 kJ/mol, 远高于Zhang和Liu^[24]计算的84 kJ/mol.

Fe催化的过氧化物中间体O-O键断裂能垒为33.9 kJ/mol, 与Lu和Lai^[53]计算的茛菪裂解加氧铁酶NOV1体系中过氧化物O-O键断裂的36.4 kJ/mol的能垒相符. 表明Fe的还原对于过氧烃中间体裂解至关重要. 体系的初始反应络合物的基态为开壳层单重态, 催化反应发生在三重态. 可能反应路径为以下几步(图8): (1) 氧合血红素的远端氧进攻底物的碳碳双键形成过氧自由基中间体; (2) 氧合血红素的近端氧进攻底物自由基, 伴随单电子还原Fe中心到+2价, 形成过氧烃中间体; (3) 过氧烃中间体的O-O在Fe(II)还原下断裂, 生成邻二醇自由基负离子和Fe(III); (4) 邻二醇自由基负离子的C-C键断裂, 同时单电子还原Fe中心到+2价, 形成产物. 整个反应决速步为第一步, 氧合血红素的远端氧进攻底物的碳碳双键, 反应能垒为82.0 kJ/mol. 本研究计算证实Lcp_{K30}反应中, 分子氧的两个氧原子都插入到了底物, 与实验观测相近的类胡萝卜素氧化酶一致^[26]. 研究结果可为深入理解Lcp_{K30}催化反应机理提供有用信息, 也为设计和理解类似断裂双键的双加氧酶提供思路.

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补充材料

图S1 DFT方法计算的过氧化物O–O键和C–C键协同断裂反应势能面以及关键结构

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Summary for “乳胶清除蛋白Lcp_{K30}催化氧气裂解聚顺-1,4异戊二烯机理”

Unraveling the mechanism of latex clearing protein Lcp_{K30} catalysed oxidative cleavage of poly(*cis*-1,4-isoprene)

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Natural rubber or synthetic rubber materials have been extensively used in our daily life and industrial production for more than 100 years due to their unique physical properties. However, a amount of rubber waste has been released into the environment, posing great challenges to the disposal of these polymers. In contrast to the disposal strategies such as combustion or stockpiling, enzyme catalyzed rubber degradation constitutes an environmentally friendly strategy.

Latex clearing protein (Lcp) is an iron-heme containing endotype dioxygenase that catalyses the oxidative cleavage of the chemically inert poly(*cis*-1,4-isoprene) which is the main component of rubber, affording the products of aldehyde and ketone. It was found that active site E148 residue is key to the reactivity of Lcp based on the mutation experiments. However, the role of E148 and the overall mechanism are still controversial. It is generally accepted that both of dioxygen atoms are incorporated into the substrate. In previous studies, two possible mechanisms of Lcp catalyzed cleavage of poly(*cis*-1,4-isoprene) are proposed. In the first one, the distal oxygen atom of Fe(III)–O₂[−] complex attacks onto the C=C double bond, leading to a three-membered epoxide intermediate. This is followed by the attack of Fe=O complex on the epoxide intermediate and subsequent C–C bond cleavage, affording the final ketone and aldehyde products. While in the second one, the proximal oxygen atom of Fe(III)–O₂[−] complex attacks onto the C=C double bond of the substrate, which is followed by the attack of the distal oxygen atom onto the substrate radical to generate a four-membered cyclic dioxetane intermediate. The final O–O as and C–C bond cleavage in the dioxetane intermediate would afford the final products.

In this study, based on the crystal structure of Lcp_{K30}, the combined molecular dynamic (MD) simulations and quantum mechanics/molecular mechanics (QM/MM) calculations are performed to elucidate the catalytic mechanism of Lcp catalysed cleavage of central double bond of poly(*cis*-1,4-isoprene). Our study shows E148 is protonated during the reaction and forms stable H bond interactions with Fe(III)–O₂[−] species. Thus, the plausible role of E148 is related to the positioning of both Fe(III)–O₂[−] species and the substrate. Moreover, it can be ruled out that the E148 acts as a base to abstract the proton from the substrate. The proposed mechanism of Lcp works as follows. Firstly, the distal oxygen of Fe(III)–O₂[−] attacks onto the carbon–carbon double bond to generate the peroxide alkyl radical intermediate. This is followed by the rebound of the proximal oxygen to the carbon radical to generate the dioxetane intermediate, with Fe(III) being reduced to Fe(II). The following O–O bond cleavage occurs via the Fe(II)-mediated reductive cleavage of O–O bond of the dioxetane intermediate, leading to the vicinal diol radical anion intermediate. Finally, a facile C–C cleavage is undergone to yield the ketone and aldehyde products. In particular, our calculations show that the direct O–O cleavage of the dioxetane intermediate in the absence of Fe(II) catalysis requires a remarkably high barrier of 160 kJ/mol, indicating the Fe(II) catalysis is vital for the further transformation of the dioxetane intermediate. For comparison, we also investigated the previously proposed mechanism involving the three-membered epoxide intermediate. However, the attack of the epoxide by Fe^{IV}=O complex is found to be highly unfavourable thermodynamically and thus can be ruled out. As such, this study may not only expand our understanding of the catalytic mechanism of Lcp_{K30}, but also provide useful information for rational design or engineering of Lcp enzyme toward the degradation of synthetic rubber materials.

latex clearing protein, catalysis, quantum mechanics/molecular mechanics methods, reaction mechanism, rubber degradation, heme

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