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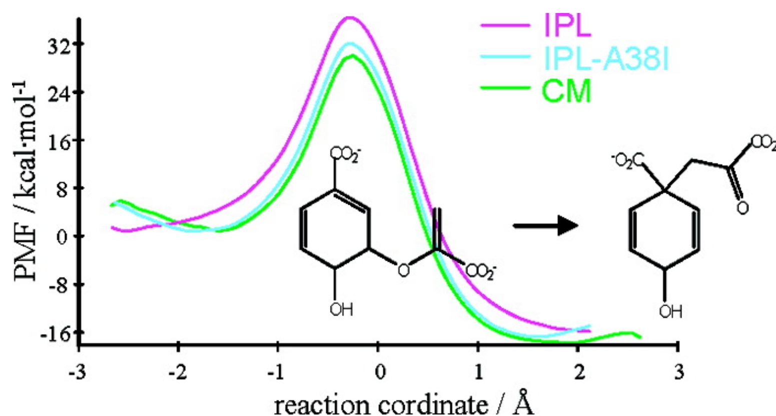

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Predicting an Improvement of Secondary Catalytic Activity of Promiscuos Isochorismate Pyruvate Lyase by Computational Design

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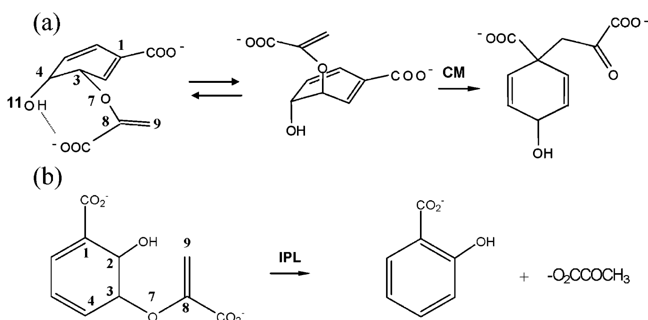
Traditional views on enzymatic activity usually remark on their high efficiency and specificity. However, it has been recently suggested that this paradigm, which has dominated thinking in this field, could be too simplistic. Many enzymes have been found to present secondary catalytic activity or catalytic promiscuity, thus being capable of catalyzing secondary reactions at an active site that was specialized to favor a primary reaction.[1−5] This promiscuity provides a raw starting point for the evolution of enzymes, as a new duplicated gene presenting low activity would provide a start for adaptative evolution.[1] In fact, new enzymatic functions can evolve in the period of years or even months, as happened recently with new synthetic chemicals or drugs.[4] According to previous studies, promiscuous activities exhibit high plasticity as they can be readily increased by means of one or few mutations, allowing the reaching the threshold to be improved under selective pressure.[1] Instead, primary activity presents a large robustness against mutations.[4] As a consequence, the active sites of these existing enzymes provide obvious starting points to engineer novel enzymes with new catalytic functions.[5]

Isochorismate pyruvate lyase (IPL), from *Pseudomonas aeruginosa*, catalyzes isochorismate transformation into pyruvate and salicylate but it also presents secondary activity catalyzing the transformation of chorismate into prephenate (see Scheme 1).[6,7] In fact, this promiscuous chorismate mutase (CM) activity was used to ascribe a 1,5-sigmatropic reaction mechanism to its native or primary activity, because chorismate mutases are well known to catalyze pericyclic reactions.[7] The recently obtained X-ray structure reveals that IPL is a structural homolog of some chorismate mutases (CMs), despite the low sequence identity.[8] Thus, this enzyme is an excellent candidate to improve its secondary activity by means of a few mutations.

There are at least two different strategies that can be used to obtain novel enzymes by means of mutations on existing enzymes. The first one is based on directed evolution, which consists of successive rounds of random mutations or recombinations followed by screening or selection.[9] This powerful tool does not require a deep knowledge of the details of the catalytic mechanism. A second strategy is the rational design that implies directed mutation on particular residues of the active site.[10] This strategy requires details about the process and the effect of the enzymatic environment on the reaction mechanism. The methods and techniques of Computational Chemistry have become a promising complementary tool to assist in the design of new enzymes. Thus, combinatorial optimization algorithms that integrate ligand docking and placement of amino side-chain rotamer libraries have been used to identify sequences that form complementary ligand-binding surfaces. Nevertheless, although impressive results have been obtained,[11,12] some drawbacks are behind these methodologies. First, the structure of the backbone of the protein remains frozen during the functional design modeling, not introducing its inherent flexibility and lacking dynamic effects; and second, the real TS of the catalyzed chemical reaction step, including the protein environment, has not been taken into account.

Scheme 1



In this communication we present an alternative computational rational approach to improve the secondary catalytic activity of enzymes, taking as a test case the IPL enzyme. Our approach is based on the use of molecular dynamic simulations employing hybrid quantum mechanics/molecular mechanics (QM/MM) methods that allow describing breaking and forming bonds.[13] This methodology provides a detailed knowledge of the transition state and the free energy profiles of the reaction taking into account the effect of the protein environment.[14]

Free energy profiles, in terms of potential of mean force (PMF), for the chorismate to prephenate reaction carried out in water and in the active site of two CMs (*Escherichia coli* and *Bacillus subtilis*, EcCM and BsCM, respectively) and IPL are shown in Figure 1. Details about the methodology are provided as Supporting Information. As expected, the catalytic efficiency of the CMs is much higher than that of IPL, keeping in mind that chorismate to prephenate rearrangement is a secondary reaction for the latter enzyme. In fact, IPL provides a free energy barrier lowering of only 2.3 kcal·mol^{-1} with respect to the reaction in aqueous solution, whereas natural enzymes are able to diminish the free energy barrier by about 8.0 kcal·mol^{-1}. The analysis of the details of the reactions shows how the chemical reaction is preceded by a pre-equilibrium between a pseudo-diequatorial and pseudo-diaxial form of chorismate, the last one being the reactant conformer closer to the pseudo-diaxial TS of the catalyzed reaction. The evolution of the dihedral angle defining the diaxial character of the substrate is presented as a function of the reaction coordinate in Figure 2. In all cases, it is observed how the diaxial character, required in the TS, is lost in the reactant state of IPL and water, whereas active sites of EcCM and BsCM constrain the substrate avoiding a complete relaxation

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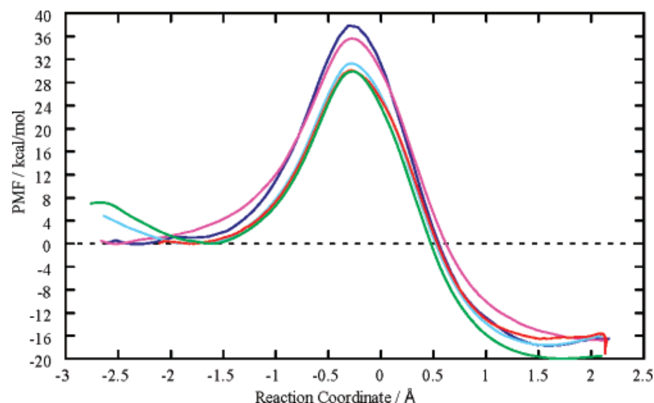

Figure 1. Free energy profiles (in terms of PMFs) for the chorismate to prephenate rearrangement obtained in different environments: EcCM (red line), BsCM (green line), IPL (purple line), IPL-A38I (light blue line), and in aqueous solution (dark blue line). The reaction coordinate is the antisymmetric combination of the interatomic distances of the breaking and forming bonds, C3···O7 and C1···C9, respectively.

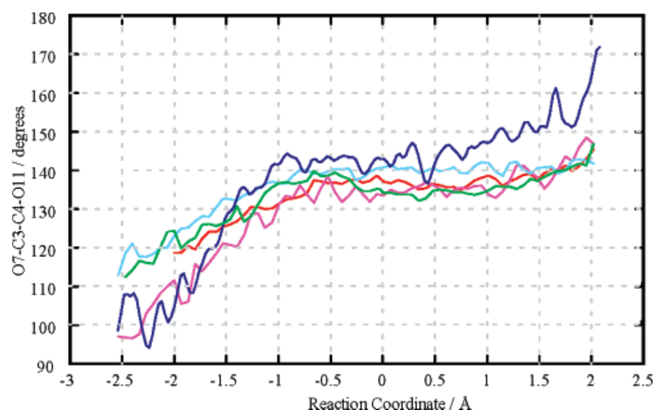

Figure 2. O7C3C4O11 dihedral angle evolution (in degrees) as a function of the reaction coordinate. EcCM (red line), BsCM (green line), IPL (purple line), IPL-A38I (light blue line), and in aqueous solution (dark blue line).

that would render a nonreactive reactant conformation.[15] It seems that IPL is not able to retain this favorable conformation from TS to reactants, probably due to the fact that the active site is not perfectly suited to accommodate the substrate of this reaction.

To understand the different behavior of IPL with respect to CMs, we are comparing the interactions of the enol pyruvyl moiety of the substrate in IPL and EcCM (which is the most closely related structure) active sites. Thus, a deeper insight into the substrate−protein pattern of interaction in EcCM reveals that there is a hydrophobic valine residue (Val35) that constrains the position of the ether bridge. The equivalent residue in IPL is a smaller alanine (Ala38) that cannot perform the same role. Thus, we decided to carried out an in silico mutation of this residue to a larger one (from Ala to Ile) and to repeat the PMF for the chorismate to prephenate reaction. The result of this mutation is that the mentioned dihedral angle evolution on the mutated IPL enzyme is closer to the CMs than to the native IPL or water (see Figure 2). This geometrical behavior is reflected in the energetics, as shown in Figure 1; the free energy barrier in the mutated IPL is 4.4 kcal·mol^{-1} lower than the native IPL, being only 2.0 kcal·mol^{-1} above the primary reaction catalyzed by BsCM.

In fact, Mayo and co-workers, after performing 19 possible aminoacids substitutions applied over 6 different positions of the engineered chorismate mutase domain of the EcCM, obtained a Val35Ile mutation that renders an increase in the k_{cat} of about 1.5 times.[16] Moreover, mutation of Val to Ala (the same residue present in IPL) reduces the k_{cat} by a factor of 2. Thus, the overall effect for a Ala35Ile mutation in EcCM is an increase of the k_{cat} by a factor of 3. Obviously, the predicted effect for the same mutation in the equivalent position in IPL (Ala38Ile) is much larger, which is consistent with the fact that this enzyme is not specialized in the catalysis of the chorismate to prephenate rearrangement. Our results are then both a prediction about the effect of a mutation on IPL and an interpretation about the success of Val35Ile mutation in EcCM. According to Figure 2, a mutation of Val by a similar but larger aminoacid at position 35 would keep the enol pyruvyl moiety in a diaxial conformation, closer to the TS geometry, and reducing then the free energy barrier. In this sense, QM/MM MD simulations of the Val35Ile variant of EcCM have been carried out, verifying that the proposed mutation increases the diaxial character of reactants by about 3°.

The experimental data of Mayo and co-workers give credence to the theoretical predictions obtained for IPL and allows being confident in our protocol. In this sense, we have been able to explain the origin of the effects observed in the native enzymes after single mutations. As a consequence, improvement of the role of promiscuous enzymes can be guided by computational protein engineering. This method can be directly applied to the design of new enzymes, and the benchmark provides a powerful *in silico* test for guiding improvements in computational enzyme design.

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Supporting Information Available: Computational details. This material is available free of charge via the Internet at http://pubs.acs.org.

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