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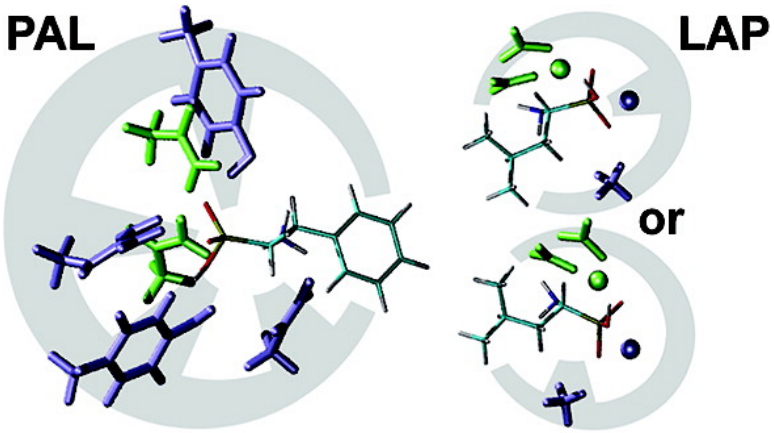

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Origins of the Activity of PAL and LAP Enzyme Inhibitors: Toward Ab Initio Binding Affinity Prediction

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Molecular recognition involving noncovalent interactions between receptor and ligand molecules determines a great variety of chemical and biological phenomena, including the catalytic and inhibitory effects of enzymes.[1] Mutual affinity of the two interacting species arises primarily from the strength and specificity of corresponding binding; thus, the full knowledge of cognitive factors and their physical nature could help to establish clear and justifiable structure−activity relationships based on the first principles.

Due to a wide range of applications, the problem of reliable yet rapid ligand affinity analysis has generated considerable interest.[2] Studies based on the comparison of stabilization energy have proven their utility in the prediction of binding affinity.[3] Since biomolecular complexes are relatively large, an accurate nonempirical analysis of interaction energy components is possible only with efficient binding energy partitioning.[4] The availability of theoretically rigorous yet computationally tractable hybrid variation−perturbation interaction energy decomposition[5] not only provides the opportunity for the study of the nature of receptor−ligand interactions but also allows one to derive and validate simplified models of inhibitory activity by the stepwise neglect of the active site residues and stabilization energy components of minor importance.

Accordingly, successful application of the aforementioned approach to a series of leucine aminopeptidase inhibitors[6] allowed, for the first time, a detailed dissection of interactions with metalloenzyme active site residues as well as systematic derivation of approximate theoretical models without losing significant correlation with experimental inhibitory activity. Herein, we extend our previous calculations to inhibitors of phenylalanine ammonia-lyase (PAL, E.C. 4.3.1.5), one of the most intensively studied plant enzymes. Its role consisting in conversion of amino acid substrate to *trans*-cinnamate locates PAL at the crossroads of primary and secondary plant metabolism and can be utilized in the control of natural product accumulation.[7] Bovine lens leucine aminopeptidase (LAP, E.C. 3.4.11.1), a metalloprotease that cleaves the N-terminal peptide bond of protein substrates, has been mentioned for comparison purposes. In both cases, phosphonic derivatives of phenylalanine (for PAL and LAP) as well as leucine (for LAP) with experimentally known inhibition constant values were investigated. Structures of six PAL inhibitors[8] considered herein are given in the Supporting Information. Since the X-ray structure of PAL from potato is unavailable, the active site of the enzyme, derived by means of homology modeling, served as a receptor for subsequent inhibitor docking followed by ligand−receptor interaction energy decomposition. The six binding site residues positioned in the vicinity of a variable part of the inhibitors were selected as follows: two asparagine ($Asn187^A$, $Asn311^A$; superscripts indicate the corresponding monomer of the PAL homotetramer) and glutamine ($Gln275^B$), as well as arginine (Arg^+281^B) and tyrosine residues ($Tyr35^A$, $Tyr278^B$; Figure S2 in the Supporting Information).

At variance with the LAP active site model[6] described previously, which contained two zinc ions (in addition to Lys^+262, Asp^-273, and Leu360), no metal ion is present in the PAL binding site. Total interaction energies reported here constitute the sums of stabilization energy obtained in a pairwise manner. Along with the variation−perturbation procedure,[5] the total interaction energy calculated at the second-order Moller−Plesset level of theory can be partitioned into the electrostatic ($E_{EL}^{(1)}$), exchange ($E_{EX}^{(1)}$), delocalization ($E_{DEL}^{(R)}$), and correlation ($E_{CORR}^{(R)}$) components:

$$E_{MP2} = E_{EL}^{(1)} + E_{EX}^{(1)} + E_{DEL}^{(R)} + E_{CORR}^{(R)} \quad (1)$$

All of these terms define the hierarchy of approximate theoretical models characterized by gradually increasing precision as well as computational cost, opening the possibility of a consistent and systematic derivation of approximate models:

$$E_{EL}^{(1)} < E^{(1)} < E_{SCF} < E_{MP2} \quad (2)$$

where the first-order Heitler−London term is defined as $E^{(1)} = E_{EL}^{(1)} + E_{EX}^{(1)}$.

The more detailed computation procedure, as well as total interaction energies between the consecutive inhibitor molecules and PAL active site residues, is collected in the Supporting Information. Besides the initial binding pocket, a model consisting of four residues ($Tyr35^A$, $Asn187^A$, $Tyr278^B$, and Arg^+281^B) identified as the most important for inhibitor binding has been considered. In contrary, out of five residues building the LAP active site model presented earlier, only $Zn^{2+}488$ and Lys^+262 were recognized as essential for relative stabilization energy.[6] It is remarkable that, among the five mostly charged LAP residues, each of the two is sufficient to determine the relative inhibitory activity. This is in sharp contrast with the generally neutral PAL binding site studied here, where a sufficiently accurate description requires consideration of the majority of constituents (four out of six residues). Neglecting two residues in the starting model ($Gln275^B$ and $Asn311^A$) results in very little qualitative change of relative stabilities (Figure 1 and Table S2 in Supporting Information). The most complete description of the binding energy obtained at the MP2 level is very closely mimicked in both models by its SCF counterpart, indicating minor influence of correlation effects. Qualitatively correct estimations of relative binding energies are gained from the $E^{(1)}$ term, especially for the six residues model. However, contrary to our earlier results[6] for LAP, where reasonable predictions were also made at the $E_{EL}^{(1)}$ level of theory and where the $E^{(1)}$ term better resembled the SCF and MP2 interaction energies,[6] further approximations do not produce a qualitatively correct estimate of relative stability in the case of PAL (Figure 1).

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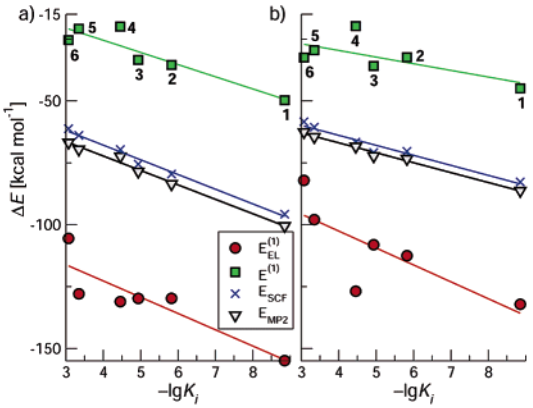

Figure 1. Binding energy at different levels of theory as a function of inhibitory activity (ref 8): (a) for the model comprising all PAL active site residues and (b) for the sum of interactions of four PAL residues (listed in text) with inhibitor molecules. Numbers of particular points correspond to inhibitors designation introduced in Supporting Information.

Table 1. Correlation Coefficients of the Relationship between Experimental Inhibitory Activity ($-\log K_i$) and the Interaction Energies at Various Levels of Theory

	PAL Active Site Model		LAP Active Site Model[a]		
method	Tyr35+Asn187+ Gln275+Tyr278+ Arg281+Asn311	Tyr35+ Asn187+ Tyr278+ Arg281	Zn488+ Zn489+ Lys262+ Asp273+ Leu360	Zn488	Lys262
E_{MP2}	0.99	0.99	0.97	0.95	0.95
E_{SCF}	0.99	0.98	0.95	0.92	0.95
$E^{(1)}$	0.92	0.69	0.94	0.91	0.93
$E_{EL}^{(1)}$	0.88	0.78	0.93	0.91	0.86

[a] LAP results are taken from ref 6.

The above discussion is further confirmed by comparison with experimental data. As demonstrated in Figure 1, excellent correlation with inhibitory activity ($-\log K_i$) is present for consecutive interaction energy terms up to electrostatic component $E_{EL}^{(1)}$. These results verify the accuracy of the PAL inhibitors' binding mode proposed on the basis of molecular mechanics as well as homology modeling methodology and confirm one of two catalytic mechanisms reported recently for PAL.[9] Additionally, these data indicate that besides the electrostatic term, exchange repulsion effects also have to be considered for reasonable reproduction of experimentally observed relationship. Table 1 provides a comparison of correlation coefficients obtained from the calculations for PAL inhibitors with analogous results for LAP.[6] Interestingly, the binding energies calculated for PAL ligands using CVFF force field resulted in the same correlation coefficient ($R = 0.88$) as that for $E_{EL}^{(1)}$ (Figure S3 in Supporting Information).

Apparently, neglecting entropic and solvation effects is justifiable when a set of similar ligands is taken into account. Reducing the PAL active site model (Figure 1b compared to 1a) does not alter the MP2 and SCF correlation coefficients, while the corresponding values for $E^{(1)}$ and $E_{EL}^{(1)}$ are affected. These findings are in contrast to earlier LAP results, revealing a significant correlation coefficient of 0.93 for $E_{EL}^{(1)}$.[6] Moreover, considering only one of the two key LAP residues (either Zn^{2+}488 or Lys^{+}262), they retain a high correlation coefficient, whereas none of the previously identified four PAL residues can be neglected without the loss of correlation. Possibly, inhibitory activity in the LAP metalloenzyme is controlled by interactions with one of a few charged residues, whereas in enzymes with a relatively neutral binding site, such as PAL, simultaneous interactions with several residues control the inhibitory activity, and exchange (steric) effects have to be considered in addition to the electrostatic interactions.

In conclusion, it has been confirmed that with the higher theory level applied to the description of intermolecular interactions, the greater the degree of correlation with experiment is observed. Significant differences were found regarding the minimal size of models representing the entire receptor properties (one and four constituents for LAP and PAL, respectively), even though the chemical nature of inhibitors in both cases was very similar. The approach utilized herein comprises a promising tool for the systematic study of molecular recognition determinants in terms of both the nature of ligand binding and the identification of the receptor residues that are crucial for specificity. As a consequence, coherent models of binding affinity prediction can be constructed ab initio, opening the way for consistent comparison of diverse receptor−ligand systems. In particular, we have demonstrated that a stringent quantum chemical approach coupled with interaction energy decomposition can also lead to simple computational models correlating with experimental ligand binding affinity and, thus, providing a sufficient estimate of the latter. Importantly, no empirical calibration is required, and the consecutive interaction energy terms derived purely from the first principles possess well-pronounced physical meaning that allows for a quantitative evaluation of the nature of the interactions. It is also worth emphasizing the significance of homology modeling and force field approaches in obtaining proper geometry of the ligand binding mode. Finally, the overall approach can be applied in the drug design process for binding affinity predictions of new ligands.

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Supporting Information Available: The total binding energies of particular inhibitor molecules at subsequent levels of theory and the details of methodology. This material is available free of charge via the Internet at http://pubs.acs.org.

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