Computation of Peptide–Protein Interactions. Catalysis by Chymotrypsin: Prediction of Relative Substrate Reactivities

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Abstract: Relative energies of the transition states for formation of acyl-chymotrypsins have been estimated by molecular mechanics for Ac-L-Trp-X and for Ac-D-Trp-X. Since the L isomer is a good substrate while the D isomer is an inhibitor and since K_s for the former and K_I for the latter are comparable, the rate difference, greater than 10^5, arises primarily from differences in energies of the transition states. This difference in activation energies amounts to 7 kcal/mol or more. We report here on results of computations based on the tetrahedral intermediates as models for the transition states. The starting coordinates were those reported by Birktoft and Blow for S195 tosyl-α-chymotrypsin. All angles and torsions of the substrate were relaxed, as were those for a segment of chain on one side of the aryl pocket. Extensive computations show that for this model the DL difference is not very sensitive to rather large variations in the force field and that van der Waals repulsions account for only 2 or 3 kcal of the D – L difference. Introduction of Coulombic terms for the three major hydrogen bond centers substantially increases the difference. The major effect, 3 kcal/mol, comes from differences in interactions between the O^- of the tetrahedral centers and the NH groups of Gly-193 and of Ser-195; the O^- of the D isomer is prevented from a close approach to these two critical NH groups. About 1.8 kcal arises from the H bond between substrate NH of the L isomers and the oxygen of Ser-214; the corresponding groups for the D isomer are more than 5 Å distant. Previous speculation had assumed that it was this latter H bond that was the dominant factor in the D – L difference. We have obtained similar results with other substrates.

Predictions of energies of intermolecular and intramolecular interactions are essential for understanding biochemistry at the molecular level or for designing new molecules having predefined properties. A great deal of progress has been achieved in predicting peptide conformations,[1-5] in the difficult problems of predicting protein folding,[6-12] and in estimating energies of crystal structures;[13-22] attempts have been made to predict binding of substrates to chymotrypsin and other enzymes.[23-26]

Predictions of enzyme–substrate specificities provide an important further area for testing computational procedures. We report here on computations for the system chymotrypsin with Ac-L-Trp-X and Ac-D-Trp-X in what we believe is the first successful computational evaluation of enzyme–substrate specificity. Prediction of relative rates to within a factor of 10 requires that $\Delta\Delta G^{\ddagger}$ be predicted to within about 1.5 kcal/mol. We have achieved accuracies of about 0.5 kcal/mol in several reaction series by carefully designed applications of molecular mechanics; this is effectively a procedure for extrapolating thermodynamic data.[27,28]

Equations 1–4 provide the framework. The steric energy

$$\Delta G_i^{\ddagger} = a + b\Delta SE_i + \text{const}_i \quad (1)$$

$$\Delta SE = SE(ts) - SE(gs) \quad (2)$$

$$\Delta\Delta G^{\ddagger} = b\Delta\Delta SE + \text{const} \quad (3)$$

$$\Delta\Delta SE = \Delta SE(2) - \Delta SE(1) \quad (4)$$

difference is computed for suitable models of the transition-state,

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(28) At the request of a reviewer we include the following description. Molecular mechanics represents the steric energy of a molecule in terms of deviations from reference bond lengths, angles, and torsions plus nonbonded interactions. The force constants and other constants are evaluated empirically from thermodynamic data. The collection of constants is called a force field and molecular mechanics calculations are also called force field calculations. The steric energy is a summation of the energy terms for all included bonds, angles, and torsions plus all included nonbonded interactions. The number of terms may reach several thousand. In the present study all torsions, most angles, and a few bonds were adjusted so as to give a minimum in the steric energy. The adjustment typically required several hundred iterations. It is critically important that all molecules are treated with rigorous consistency. To ensure that each calculation included exactly the same nonbonded terms all 43 000 terms were included in evaluation of the reported steric energies. The double-difference ΔΔSE values are relatively insensitive to the constants used in the force field. In the present study we assumed that ΔSE = 0 for the reactants on the grounds that the binding constants have been reported to be nearly equal; hence ΔΔSE = ΔSE for the transition states.

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SE(ts), and of the ground state. If two reactions are closely similar, then the constant of eq 3 will perhaps be negligibly small; the constant represents such factors as polar effects, solvation, and entropy. The latter two tend to cancel in the double difference of eq 4. The linear free-energy expression, eq 1, is a generalization of eq 3. If $\Delta\Delta SE$ is equal to $\Delta\Delta G^{\ddagger}$, then $b = 1$ and const = 0. Usually in the calculation of steric effects for a series of reactions the value of b departs somewhat from unity and thus a and b of eq 1 are constants to be adjusted.[27] In the present preliminary computations we assume $b \approx 1$ and const = 0. The key assumption is that the reaction-specific terms, const$_i$ must either be calculable or else be negligibly small. The success of our approach is enhanced by the important finding that predicted $\Delta G_i^{\ddagger}$ of eq 1 is not very sensitive to moderate variations in the force field.[27] This is, of course, a critical factor since there is no way at present to obtain precise values of parameters for transition states. What is essential is to assure absolute accuracy in the mathematical treatment so as to apply precisely the same computation to each molecule. In the present study we assume that SE(gs) is the same for the two enantiomers, on the basis of the experimental findings. The calculated steric energies are nearly the same, but the conformational possibilities have been incompletely explored. For a DL pair, then, we assume that $\Delta\Delta SE = SE(ts_D) - SE(ts_L)$.

We emphasize the power of the Eyring methodology as represented in eq 1–3. It is not necessary to be concerned with intermediate states between reactants and products nor with the complexities of reaction trajectories.[29-31] We are not concerned about details as to how bonds are formed or broken so long as we are careful to choose appropriate models for ground state and for transition state.

Treatment of enzyme–substrate systems poses great problems of logistics. An enzyme typically has 3000 or more atoms, and even the greatly simplified model we have treated involves more than 40 000 nonbonded interactions. Therefore, choices of enzyme, of substrates, and of appropriately simplified models are all matters of critical importance.

Chymotrypsin has many advantages.[32] There are three independently determined sets of X-ray coordinates.[33-44] Chymotrypsin acts upon a wide range of substrates, thus providing a wealth of quantitative examples.[32,35,36,45,46] The extensive studies

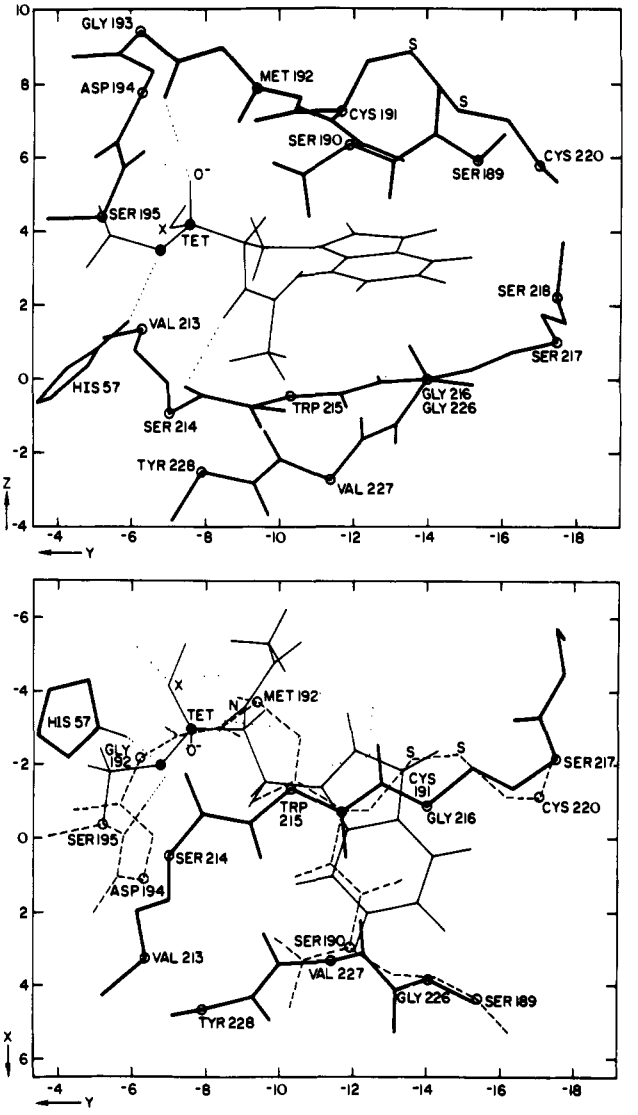

Figure 1. Orthographic projections of Ac-L-Trp-X as tetrahedral intermediate at the active site of chymotrypsin. Coordinates have been rotated so as to provide a view more or less vertically down into the specificity pocket.[57] The upper view looks down into the pocket, the lower view looks across the pocket. The α-carbon atoms of the enzyme residues are circled. Dotted lines show the critical hydrogen bonds. The dotted partial figure of an indole ring in the bottom view shows the position of HCO–TrpO$^-$ reported by Steitz et al.[33]

provide a clear definition of the mechanism of action and reliable rate estimates for many substrates.[32,47-54] Relatively little movement of the peptide chains upon binding of inhibitors or of pseudosubstrates assures that conformations of the system are relatively well-defined.[33,35,40,55]

We report here on the substrate Ac-L-Trp-X and its D isomer; the choice of X group is unimportant for computational purposes

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at this stage. We used $-OCH_3$ simply to check if the X group would remain out of the way. The rate data pertain to X = NH_2. The L isomer (X = NH_2) is a good substrate while the D isomer is an inhibitor, yet both bind comparably well since $K_s(L) \simeq K_I(D)$.[46,56] The rate difference is of the order of 10^5 or more so that $\Delta\Delta G^{\ddagger} > 7$ kcal/mol. An additional advantage of this substrate is the large indole ring which tends to limit conformational possibilities.

As the starting reactant we chose the enzyme–substrate complex. As model of the transition state on the path to acyl enzyme we chose the tetrahedral intermediate Figure 1. Our ester studies have shown that the tetrahedral intermediate is a good representation of the transition state for both acid-catalyzed and for base-catalyzed hydrolysis.[27,57] It should be equally valid for representing steric effects in enzymatic catalysis. These several choices tend to make the constant in eq 3 small. Thus the double difference in solvation will be small since both ground states and both transition states have nearly identical solvent exposure. Differential entropic effects concern differences in librational entropy of the two transition states and of the two reactant states (enzyme–substrate complexes). For large substrates the configurations are relatively restricted. Entropic effects have been critically evaluated for a series of cyclization reactions that are less restricted; differential effects prove to be rather small.[27b] In any event we have assumed that for the present substrates differential solvation and differential entropic effects are zero. This will be valid if they are either negligibly small or if they parallel $\Delta\Delta$SE.

For our computational model we have simplified the enzyme to the following active site residues. Residues held fixed were Cys-42-Gly-43, Ala-55-Ala-56-His-57-Cys-58-Gly-59, Asp-102, Gly-142-Leu-143, Val-213-Ser-214-Trp-215-Gly-216-Ser-217-Ser-218-Thr-219-Cys-220-Ser-222, and Gly-226-Val-227-Tyr-228; the movable residues were Ser-189-Ser-190-Cys-191-Met-192-Gly-193-Asp-194-Ser-195-Gly-196. The N of Ser-189 and the C of Gly-196 were anchored to prevent any tendency to drift apart. The residues comprise 30 of the possible 245 residues. Even if memory and computing time permitted us to include the total enzyme, the model would still be incomplete since the solvent medium and pH are known to influence the conformation. Our coordinates are based on the widely quoted Birktoft and Blow study of S-195 tosyl-α-chymotrypsin.[34,44] The hydrogen atoms were added.

We have investigated the following specific questions. (1) Assuming that the Birktoft–Blow coordinates provide a precise definition of the active site, what difference is there in van der Waals energies of transition-state models for Ac-L-Trp-X and Ac-D-Trp-X? Consideration of physical models suggests that the NH–Ac group of the D isomer might interfere with the Met-192 of the pocket of Figure 1. The success of the hard-sphere Ramachandran[1] computations in predicting peptide conformations suggests that van der Waals repulsions might account for the D – L differences. In fact the computations reveal no significant van der Waals differences in the D and L tetrahedral models; the D-NH–Ac group simply rotates slightly into a nearly vertical position with respect to the pocket (Figure 1) so that there is no interference. In this calculation the main chain atoms were held at the X-ray positions. The substrate and side chains of Ser-195 and of His-57 were relaxed.

(2) If the pocket is caused to close down on substrate, then will van der Waals differences appear? One side of the active site pocket (top side in Figure 1, upper view) consists of a chain that undergoes an appreciable shift as chymotrypsinogen is converted to chymotrypsin.[32] A possible function of the bulk of the enzyme is to provide a buttressing effect, a spring-like tension that tends to close the pocket as well as to maintain its integrity. A subsidiary effect of relaxing this chain is to make partial allowance for uncertainties in X-ray coordinates. The published coordinates involve a considerable number of bad contacts (amounting to van der Waals repulsions totaling more than 100 kcal/mol over this small region of the enzyme). Even such large energies as these can be reduced by quite minor adjustments of the atomic positions; they do not interfere with estimates of D – L differences because they cancel exactly if the atomic coordinates remain fixed.

With the fixed-enzyme coordinates used in connection with question 1, there appears to be just one single conformation of the substrate. Movement of the peptide chain requires introduction of restraints, one across the pocket to represent the buttressing effect and possibly one to keep the substrate from rising out of the pocket; the latter can be interpreted as a representation of solvation forces. The number of variations now available can lead to families of local minima. Even though we investigated a considerable range of values for the two restraining forces, some of which caused unrealistic distortions, we could not find multiple conformations that differed significantly in energy or in structure. In fact the D – L differences remained relatively constant throughout while total energies for the several choices of restraining forces varied over tens of kilocalories.

We report results on the basis of the values of restraining forces that have energies of 2 kcal or less and $d(SE)/dr$ of about 7 kcal/(mol Å) for the pocket restraint and half as much for the pull-down restraint. The substrate and the enzyme residues listed above were relaxed in this computation.

The overall D – L difference in van der Waals repulsions with this model of the transition state amounts to 2.6 kcal/mol, the L isomer having the lower energy; 0.5 kcal of this difference arises from the above described restraining forces. We were unable to increase this van der Waals difference appreciably with any plausible values of restraining forces or by varying the positions of application. The movement of the enzyme chain is relatively slight, about 1 Å at the position of greatest change.

(3) What is the contribution of Coulombic forces? It has been clear from the X-ray studies that hydrogen bonds are formed favorably for a proposed tetrahedral intermediate.[34] The H of the NH–Ac group of the L isomer forms a H bond to the O of Ser-214; such a bond cannot be formed by the D isomer. The "orienting influence" of this bond is generally regarded as of great importance. Also important for both isomers are H bonds betwen the O^- of the tetrahedral intermediate and the NH groups of Ser-195 and of Gly-193.

Coulombic forces are typically incorporated in a force field to represent dipolar interactions as well as such polar interactions as those to O^-. They must be treated with circumspection in computation because they can be very large; a Coulombic interaction can exceed 10 kcal/mol where the corresponding van der Waals interaction is of the order of 0.2 kcal/mol or less. We have therefore adopted the expedient of including those specific sets of Coulombic interactions at the three most important hydrogen-bonding sites while all others are omitted on the expectation that they provide a residual Coulombic field that largely cancels in the double differences. We verified this point by including all terms in some computations.

Those specifically included in the present calculations were as follows: tetrahedral center with the three peptide bonds, those of Met-192–Gly-193 (includes H bond O^- to NH of Gly-193), those of Asp-194–Ser-195 (includes H bond O^- to NH of Ser-195), and those of Ser-214–Trp-215. Also incorporated were interactions of the amide center of the substrate with each of these same three peptide bonds; for the L isomer these latter include the key H bond between the NH of substrate and the O of Ser-214. The remote pairs of interactions in this set provide a sample of the magnitudes of the neglected Coulombic interactions.

Results are summarized in Table I for minimizations carried out in the presence of the Coulombic interactions summarized above. Details of the force field and of the geometry of the L

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(57) Coordinates ($\bar{x}$, $\bar{y}$, $\bar{z}$) shown in Figure 1 and in the supplementary material are derived from those published by Birktoft and Blow (x, y, z)[34,44] by the following transform in order to provide a viewpoint looking directly into the specificity pocket.

$$\begin{vmatrix} 0.10576 & -0.61342 & 0.78264 \\ -0.64953 & 0.55334 & -0.56350 \\ -0.75295 & -0.56350 & -0.33991 \end{vmatrix} \times \begin{vmatrix} x - 14.2 \\ y - 5.3 \\ z - 13.1 \end{vmatrix} = \begin{vmatrix} \bar{x} \\ \bar{y} \\ \bar{z} \end{vmatrix}$$

Table I. Sources of Energy Differences in Models of Transition State for Chymotrypsin with L- and D-Ac-Trp-X

	Ac-L-Trp-X	Ac-D-Trp-X	diff
bonded	19.5[a]	19.5	0.0
Tet to M192-G193[b]	−5.3[c]	−4.7[c]	−0.6[c]
Tet to D194-S195	−5.2[c]	−3.2[c]	−2.2[c]
Tet to S214-W215	0.6[d]	0.8[d]	−0.2
amide to M192-G193	−0.1[d]	0.2[d]	−0.3
amide to D194-S195	−0.1[d]	0.1[d]	0.2
amide to S214-W215	−1.8[e]	0.0[d]	−1.8
other van der Waals	−113.6	−111.0	−2.6
total nonbonded[f]	−125.5	−117.8	−7.7
net bonded plus nonbonded	−106.0	−98.3	−7.7

[a] Absolute values of the energies (kcal/mol) depend on details of the force field and on details of the model; the differences are relatively independent of these choices. Bonded includes the total effect of all distortions in substrate or in the adjusted enzyme residues; most of the energy derives from the numerous minor distortions of the enzyme, none of which individually is large. The total nonbonded energy is normal for the number of atoms involved and with the force field used. See footnote *f*. The C(tetrahedral)–O^{γ}(Ser-195) bond was treated as a normal C-O bond with 1.45 Å reference distance and a force constant of 4.4 mdyn/Å; the adusted value was 1.46 Å. [b] Tet value includes interactions of partial charges on the four atoms X, O of Ser-195, O^-, and the tetrahedral carbon with partial charges on the peptide CO of Met-192 and the peptide NH of Gly-193. Similiar interactions are included for the Asp-194–Ser-195 peptide bond and for the Ser-214–Trp-215 peptide bond. [c] These are net effects of hydrogen bonding to the O^- of the tetrahedral intermediate with allowance for all other Coulombic terms listed in footnote *b*. [d] These terms illustrate the residual effects of more remote charged groups. The values do not change appreciably with the minor adjustments in geometry needed to go from a poor fit to an energy minimum. [e] This is the net effect of the hydrogen bond between NH of the X-AcNH group of Trp to the O of Ser-214. [f] Total for some 45 000 nonbonded interactions plus the included Coulombic terms.

isomer (Figure 1) are presented in the supplementary material. We tried the Hagler and Lifson convention of using a zero van der Waals repulsion for the H···O nonbonded part of the H bond. This allowed one of the H bonds to collapse (O^- to H of Gly-193); incorporation of a repulsive H···O force about 30% of the normal value gave nearly the same H···O distances with the other H bonds as did the zero force constant and also gave a normal distance for the errant H bond. The energy differences reported in Table I, on the basis of the H bond distances of about 2.2 Å for the L isomer, show an energy in favor of the L isomer of 7.5 kcal/mol. At present we must consider this as an approximation since we can increase this difference by adjusting the repulsive term to allow formation of a shorter H bond for the L isomer. The D − L energy difference remains if all possible Coulombic terms are included.

We may summarize the conclusions as follows. Insofar as the Birktoft–Blow coordinates provide a valid description of the geometry at the active site of α-chymotrypsin, it is the Coulombic factors that play the dominant role in differentially stabilizing the transition state for the L-Ac-Trp-X over that of the D isomer. There is one residual repulsive van der Waals interaction between the D-NH group and the enzyme. Adjustments necessary to minimize repulsive D-NH interactions have caused residual van der Waals repulsions to become broadly dispersed; indirectly the interactions prevent optimal H bond formation to the developing O^-, thus leading to a significant D − L difference in Coulombic energies. Our studies with other substrates have given results in accord with the tryptophan results. We anticipate that further detailed computations based on other models, substrates, and X-ray data will yield a more comprehensive understanding of the quantitative factors underlying enzymatic catalysis. Treatment of less rigid substrates will also require an appropriate analysis of entropic factors.

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Supplementary Material Available: Tables of a description of the molecule, parameters, energies, bond distances, cartesian coordinates, and atom types, moveable atom sets for α-chymotrypsin and substrates and chymotrypsin-fixed active site atoms, and a data summary (37 pages). Ordering information is given on any current masthead page.