Quantitative Measures of Molecular Similarity: Methods to Analyze Transition-State Analogs for Enzymatic Reactions

CAREY K. BAGDASSARIAN AND BENJAMIN B. BRAUNHEIM
Department of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, New York 10461

VERN L. SCHRAMM
Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York 10461

STEVEN D. SCHWARTZ
Department of Physiology and Biophysics and Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York 10461

ABSTRACT

A formalism is presented for quantifying the similarity between any two molecules. The chemical descriptor used for comparison is the molecular electrostatic potential at the van der Waals surface. Thus, both the spatial properties of a molecule and its chemical features are captured in this approach. For molecules that are geometrically alike, the most useful similarity measure stems from orienting the two species so that their physical surfaces are aligned as well as possible, without regard to chemical patterns. After this alignment is achieved, a single measure sensitive to the spatial distribution of the electrostatic potential is used to rank the electronic similarity. Molecular similarity measures are applied to the enzyme systems AMP deaminase and AMP nucleosidase in order to understand quantitatively why their respective transition-state inhibitors bind more tightly than do their substrates.

Introduction

In an enzymatically catalyzed biochemical reaction, the transient, high-energy transition-state structure is the species along the reaction coordinate most tightly bound to the enzyme. The observed rate enhancement over the analogous uncatalyzed reaction for the conversion of substrate to product reflects that the transition state is typically bound 10^{10}–10^{15} times more tightly to the

International Journal of Quantum Chemistry: Quantum Biology Symposium 23, 1797–1804 (1996)

CCC 0360-8832 / 96 / 081797-08

enzyme than is the substrate [1]. Therefore, if a chemically stable transition-state analog can be designed, it would out-compete the substrate in binding to the active site and would serve as a powerful enzyme inhibitor. Metabolic pathways leading to disease caused by pathogenic organisms frequently involve foreign enzymes introduced into the host. Targeting these pathogen-specific enzymes with powerful transition-state-like inhibitors can shut down the pathways, underscoring the importance of the development of transition-state analogs. For example, the organism *Vibrio cholerae*, the causative agent of cholera in humans, uses an enzyme which modifies geometrically and electrostatically its substrate NAD^+ into the transition-state structure, the target for inhibitor design. The assumption is made that any analog showing geometric and electrostatic similarity to the transition state enjoys interactions with enzymatic residues in the active cleft similar to those leading to transition-state stabilization. By this reasoning, the more similar an analog to the transition state, the more tightly it will be bound. In this work, we introduce a quantitative measure of molecular similarity which scores the likeness of substrate or putative inhibitors to the transition state of an enzymatic reaction and thereby ranks their binding constants to the enzyme. The method will prove useful for rational drug design.

Although our formalism can be used to quantify the similarity of any two molecules, we are most interested in the development of transition-state analogs. For any enzymatic reaction, then, detailed knowledge of the transition-state structure is required. Such information is becoming available through kinetic isotope effect experiments [2]. As a test of the method, the measure of molecular similarity is applied to the enzyme systems AMP deaminase and AMP nucleosidase for which the transition-state structures of their common substrate AMP are known [3]. The needed biochemistry will be sketched below, and a full description of the biochemical relevance of our work is offered in [4].

Two molecules are scored for similarity by a quantitative comparison of their molecular electrostatic potential distributions at their van der Waals surfaces. Application of the algorithm proceeds in two phases. Because substrate molecules, the transition-state structures derived from them, and the transition-state analogs must share global geometrical similarities, the first requirement is that the two molecules to be tested are oriented with respect to each other for maximal overlap of their geometrical surfaces. This is done through a purely geometric similarity measure called S_g. The first molecule, usually the transition state, is placed with its center at the origin of the coordinate system. The second test molecule is likewise centered, but is allowed to undergo center-of-mass rotations until S_g is maximized. Because the geometries of the molecules are so similar, no center-of-mass translations are required for the global geometric optimization. After this best relative orientation is achieved, a second measure S_e, sensitive to both electrostatic and geometrical features, is used to score the similarity of the two molecules. It is S_e that is well correlated with the binding constant of an inhibitor or substrate to the enzyme. For any enzyme system with a known transition-state structure, S_e is used to score the similarities of the substrate and the inhibitors with the transition state. If an inhibitor is to be bound more tightly than is the substrate, it should be more similar to the transition state than is the substrate.

As stated above, the molecular electrostatic potential calculated at the van der Waals surface of a molecule is the chemical descriptor used in this work. This surface is known to reveal regions of electrophilicity, nucleophilicity, and hydrophobicity, as well as hydrogen-binding sites and so is most pertinent to understanding the problem of molecular recognition [5–8]. Previous workers using molecular electrostatic potential surfaces have projected them onto surfaces of lower complexity [9], mapped them to a single autocorrelation vector for each molecule prior to similarity comparison [10], and developed group theoretic methods for their study [11]. Our formalism compares two molecular surfaces directly without need for any initial mappings, i.e., the integrity of the molecular electrostatic potential surface of a molecule or transition state is maintained. We offer, in addition, a procedure for a physically motivated mapping of the substrate or inhibitor surface onto that of the transition state. In this way, all geometrical differences between molecules can be erased, leaving as the sole distinction the electrostatic potentials: the data required for specification of a molecule is greatly reduced. This mapping preserves the similarity rankings calculated from the unmanipulated surfaces.

Quantitative Similarity of Molecular Electrostatic Potential Surfaces

Let $\varepsilon_A(\mathbf{r})$ represent either the electron density or the electrostatic potential around the spatial point $\mathbf{r}$ for molecule A, with $\varepsilon_B(\mathbf{r})$ the similar quantity for molecule B. A family of molecular similarity measures [12] was based on the construct

$$R_{AB} = \frac{\int \varepsilon_A(\mathbf{r})\varepsilon_B(\mathbf{r})\, d\mathbf{r}}{\sqrt{\int \varepsilon_A(\mathbf{r})\varepsilon_A(\mathbf{r})\, d\mathbf{r}}\sqrt{\int \varepsilon_B(\mathbf{r})\varepsilon_B(\mathbf{r})\, d\mathbf{r}}}. \quad (1)$$

It is assumed that the two molecules A and B are properly oriented with respect to each other. The integrations span over all space within and exterior to the van der Waals surfaces (though densities in the molecular cores can been excluded, see above references). If two molecule are identical, clearly $R_{AB} = 1$; if they differ in their distributions of either electron density or electrostatic potential, then the measure is less than 1.

For the present work, the molecular comparison is specialized to the van der Waals surfaces of molecules A and B. Assume that we have at our disposal electrostatic potential values only at the molecular surfaces. In general, because molecules A and B do not have identical surfaces, a point $\mathbf{r}$ on the surface of A need not have its counterpart on the surface of B. This means that a point-by-point integration as implied by Eq. (1) does not have any meaning here. The following similarity measure is therefore proposed:

$$S = \frac{\int\int \varepsilon_A(\mathbf{r}_A)\varepsilon_B(\mathbf{r}_B)\exp(-\alpha r_{AB})\, d\mathbf{r}_A\, d\mathbf{r}_B}{\sqrt{\int\int \varepsilon_A(\mathbf{r}_A)\varepsilon_A(\mathbf{r}_{A'})\exp(-\alpha r_{AA'})\, d\mathbf{r}_A\, d\mathbf{r}_{A'}}} \times \frac{1}{\sqrt{\int\int \varepsilon_B(\mathbf{r}_B)\varepsilon_B(\mathbf{r}_{B'})\exp(-\alpha r_{BB'})\, d\mathbf{r}_B\, d\mathbf{r}_{B'}}}. \quad (2)$$

In the numerator, the integration over $\mathbf{r}_A$ is constrained to the surface of A, and $\mathbf{r}_B$ is likewise on the surface of B. r_{AB} is the distance between $\mathbf{r}_A$ and $\mathbf{r}_B$ and is defined as $\mathbf{r}_{AB} = \|(\mathbf{r}_A - \mathbf{r}_B)\|$. For any point on the surface of A, the integration is over all points on the surface of B with the product $\varepsilon_A\varepsilon_B$ modulated by an exponentially decreasing factor in the distance between the two points under consideration. If points $\mathbf{r}_A$ and $\mathbf{r}_B$ are spatially close, their product is more important for similarity scoring than if the points were far apart. As such, a point on A explores a neighborhood on B for similarity comparison. Because two molecules are never identical—a transition-state analog can never match exactly the geometry of the unstable transition-state structure—and because there are thermal fluctuations and some degree of elastic flexibility in the positions of the contacts between enzyme residues and the bound species, it is advantageous not to restrict the similarity scoring only to that point on B closest to a given point on A. α defines the length scale for the exponential decay. In the normalizing numerator, two identical copies of molecule A (or of B) are perfectly superimposed and all points $\mathbf{r}_A$ on one copy are compared with all points $\mathbf{r}_{A'}$ on the other.

If ε_A and ε_B represent the electrostatic potentials, Eq. (2) is applied as is. If they are, instead, values of the electron density on the van der Waals surface, ε_A and ε_B are simply the same constant (approximately 0.002 electrons/bohr^3 [5]), and this leads to a second, purely geometrical measure:

$$S' = \frac{\int\int \exp(-\alpha r_{AB})\, d\mathbf{r}_A\, d\mathbf{r}_B}{\sqrt{\int\int \exp(-\alpha r_{AA'})\, d\mathbf{r}_A\, d\mathbf{r}_{A'}}} \times \frac{1}{\sqrt{\int\int \exp(-\alpha r_{BB'})\, d\mathbf{r}_B\, d\mathbf{r}_{B'}}}. \quad (3)$$

It is simple to examine how these two measures work qualitatively. The maximum value of S' is unity, occurring when the two molecules under consideration are identical and oriented so that their surfaces are perfectly coincident. If one of the copies is now deformed into a different surface, the exponential terms in the numerator clearly become smaller since points originally occupying the same spatial position are now moved away from each other. The result is that $S' < 1$. S' is used to orient two molecular surfaces for maximal geometrical similarity. As discussed in the Introduction, place one molecule (the transition state) at the origin of the coordinate system, and place the test molecule (inhibitor or substrate) with its geometric center at the origin as well. (The meaning of this geometric center is clarified below.) The test molecule is rotated until S' is maximized. Now, an electrostatic scoring is possible via application of S for the relative orientation achieved through S'. In Eq. (2), ε_A and ε_B define the electrostatic potentials on the surfaces of the molecules, but note that

S includes geometrical sensitivity through the exponential. If two molecules are geometrically and electrostatically similar, like-signed regions of electrostatic potential will be closely oriented, leading to a value of S close to unity. As the molecules become more dissimilar, oppositely signed regions of potential may overlap, decreasing the value of the measure. In summary, for two identical molecules, both S and $S' = 1$. As molecular electrostatic and geometrical dissimilarity increases, S and S' decrease continuously from unity.

Algorithm for Similarity Scoring

The spatial distributions of electron density and electrostatic potential for a molecule are calculated with the *Gaussian* 94 package (Gaussian, Inc.) at the Hartree–Fock level with the STO-3G basis. Calculation with the 6-31 + G** basis does not change any conclusions. The outputs are space-filling three-dimensional matrices of numbers reflecting the discretization in the x-, y-, and z-coordinates. To specialize to the van der Waals surface, an algorithm was written to scan the electron density matrix for spatial points having densities in the neighborhood of 0.002 electrons/bohr^3. This value correlates well with the surface containing 95% of the electron density [5]. Although the choice of this density is arbitrary, its use has led consistently to a correct ranking of enzymatic binding constants while very low or high densities can lead to spurious results. The spatial coordinates of this collection of points are recorded, and the associated electrostatic potential at each point is extracted from the potential matrix. The set $\{x_i, y_i, z_i, \varepsilon_i\}$ defines the van der Waals surface of a molecule, where ε_i is the electrostatic potential of the *ith* point. Increasing the neighborhood around the 0.002 electrons/bohr^3 density target leads to extraction of more points from the *Gaussian* matrix. However, too large a spread gives spatial distortion as points flanking the needed surface are accepted. Typically, 17 points per atom gives a good surface coverage as can be checked on a visualization package such as *AVS* (Advanced Visual Systems, Inc. and Molecular Simulations, Inc.). Visualization is also indispensable for checking the relative orientation of two surfaces. To find the geometrical center of molecule, it is assumed that each surface point carries the same mass and the usual center of mass is calculated. Two molecules for similarity scoring are placed with their center of masses at the coordinate origin.

This scheme gives for any molecule—substrate, inhibitor, or transition state—its van der Waals surface, and each molecule has a unique surface. Similarity comparisons are performed for these surfaces *without* any mapping to simpler surfaces, and this is a strength of our method. It is, nonetheless, instructive to consider also the results of similarity comparisons under a simple mapping, the utility of which is to reduce the data set required for description of a molecule. The experimentally known information is the transition-state structure. No knowledge of the enzymatic active cleft is assumed. However, the shape of the transition state offers a good idea of the shape of the active site. We can say that, roughly, the substrate or inhibitor must "fill" as best it can the volume defined by the transition-state surface. The mapping is as follows: (a) Align geometrically the inhibitor or substrate to the transition state; (b) for each point on the transition state, find the nearest point on the test molecule and record the electrostatic potential of that test molecule point; and (c) create a new surface for the *test* molecule by assigning to each spatial point on the *transition-state* surface the electrostatic potential of the nearest point on the *test* molecule. Therefore, transition states, substrates, and inhibitors are now represented by the same geometrical surface—that of the transition state—but the electrostatic potentials at these surface points for the substrates and inhibitors are derived by projection from the electrostatic potentials on their original surfaces. The electrostatic potential of the test molecules "fills" into the transition-state surface, which is a good model for the active site. The usefulness of such a mapping is twofold. First, the robustness of the similarity calculations can be tested under a biochemically reasonable transformation of surfaces. Second, as implied above, all molecular species can be represented by the same set of coordinates for their surfaces, and so the only difference among transition state, substrate, and inhibitor is in their respective electrostatic potentials at these points. Thus, these molecules are differentiated without geometrical considerations, and this data reduction will prove to be useful in future work. However, we stress that this mapping is not at all required for similarity scoring with our algorithm.

Because the van der Waals surface of a molecule is now represented by a discrete collection of points, the working forms of Eqs. (2) and (3) are

$$S_g = \frac{\sum_{i=1}^{nA}\sum_{j=1}^{nB}\exp(-\alpha r_{ij}^2)}{\sqrt{\sum_{i=1}^{nA}\sum_{j=1}^{nA}\exp(-\alpha r_{ij}^2)}\sqrt{\sum_{i=1}^{nB}\sum_{j=1}^{nB}\exp(-\alpha r_{ij}^2)}} \tag{4}$$

and

$$S_e = \frac{\sum_{i=1}^{nA}\sum_{j=1}^{nB}\varepsilon_i^A\varepsilon_j^B\exp(-\alpha r_{ij}^2)}{\sqrt{\sum_{i=1}^{nA}\sum_{j=1}^{nA}\varepsilon_i^A\varepsilon_j^A\exp(-\alpha r_{ij}^2)}} \times \frac{1}{\sqrt{\sum_{i=1}^{nB}\sum_{j=1}^{nB}\varepsilon_i^B\varepsilon_j^B\exp(-\alpha r_{ij}^2)}}. \tag{5}$$

nA and nB are the number of surface points on molecules A and B, respectively. ε_i^A and ε_j^B refer to the electrostatic potentials at point i of A and j of molecule B. r_{ij} is the distance between these points. Note that the distance is now squared to reduce computation time. In the denominator, r_{ij} refers to the distance between two points on the same molecule. In accord with the above discussion, S_g is the purely geometric measure and is used to position the two molecules with respect to each other. The target molecule is fixed, and the other is reoriented by application of the rotation matrix to each of its surface points. For each new rotation, S_g is calculated and its maximum value defines the best relative orientation of the two molecules; 100,000 random reorientations more than adequately covers rotation space. The time required for the search is reduced by, in addition to squaring r_{ij}, calculating $\exp(-\alpha r_{ij}^2)$ only if $\alpha r_{ij}^2 \leq 4.5$ for a pair of points. This means that points for which $\exp(-\alpha r_{ij}^2) \leq 0.011$ are ignored, and this changes only negligibly the final relative orientation. Another approach is to use a simulating annealing scheme to optimize S_g. After this initial stage, S_e is applied either directly to calculate the similarity of the electrostatic spatial distributions of the two molecules or, alternatively, the substrate and inhibitor geometries are mapped as described above onto the transition state's prior to scoring via S_e. Finally, the algorithm is used with different values for α, and the similarity ranking will be shown to be robust to changes in this parameter. Though S_e and S_g share mathematical features with the alignment function used by Kearsley and Smith and by Klebe et al. [13], these workers aligned molecules based on matches of atomic partial charges and van der Waals radii. In the present work, a full quantum mechanical description of the biochemically relevant molecular surfaces is used for the similarity rankings.

AMP Deaminase and AMP Nucleosidase

AMP (adenosine monophosphate) deaminase catalyzes the hydrolysis of AMP to inosine monophosphate and ammonia. The structure of the substrate AMP is shown in Figure 1. Note that the ribose portion of the molecule has been replaced by a $-CH_3$ substituent at N-9. The sugar moiety is not involved in the reaction and is ignored for the substrate, as well as for the transition

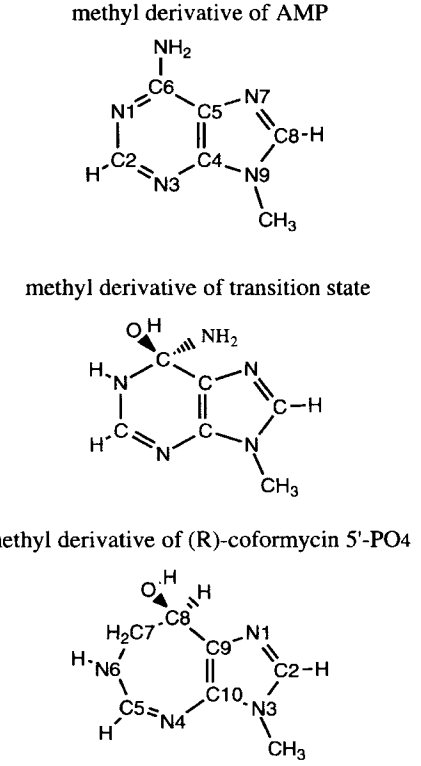

FIGURE 1. Substrate, transition state, and inhibitor for AMP deaminase reaction. Note the methyl subsituent replacing the ribose 5'-PO_4 residue on each.

state and for the inhibitors of this enzyme system. The experimentally determined transition state for AMP features nucleophilic attack by a hydroxyl group at C-6 and protonation at N-1 of AMP (Fig. 1), rendering the transition state different both in geometry and in electrostatic character from the substrate. The powerful transition-state inhibitor (*R*)-coformycin 5′-PO_4 is bound 5×10^7 times tighter to the enzyme than is the substrate and is expected to show greater similarity to the transition state. Indeed, examination of the figure shows a hydroxyl group at C-8 and a hydrogen at N-6, which confer to it features found at the analogous C-6 and N-1 loci of the transition state. On the other hand, (*S*)-coformycin 5′-PO_4 with incorrect stereochemistry at C-6 is bound only slightly tighter to the enzyme than is AMP. As this poor inhibitor is simply the mirror image of (*R*)-coformycin, its structure is not shown.

The three-dimensional geometries for these molecules have been built through energy minimization (at the Hartree–Fock level with an STO-3G or higher basis set) on the parent experimental crystal structures for AMP and the inhibitors. For transition-state modeling, certain bond lengths and angles are fit to the experimental kinetic isotope effects and are constrained during geometry optimization. Full discussion and visualization of the resulting molecular electrostatic potential surfaces can be found in [2, 4, 14]. It is these surfaces which are to be represented by a collection of points $\{x_i, y_i, z_i, \varepsilon_i\}$. For similarity scoring, we test the substrate, the (*R*)-inhibitor, and the (*S*)-inhibitor against the transition-state structure.

AMP nucleosidase is the second enzyme system that we explore. Here, as shown in Figure 2, AMP is the substrate, and the transition-state inhibitor formycin 5′-PO_4 is bound 2600 times tighter. The enzyme catalyzes the cleavage of the C-1′—N-9 bond. It is expected that S_e for the inhibitor–transition-state pair, as compared to its value for AMP scored against the transition state, will be larger (closer to unity). As seen in the figure, the transition state is derived from AMP by nucleophilic attack on the substrate at C-1′ and protonation at N-7. This protonated state is mimicked by the hydrogen at N-7 of formycin. The three-dimensional potential surfaces of these three species are reproduced in [4, 15].

In summary, the procedure for similarity scoring is as follows: (1) extract from the *Gaussian* output the coordinates and electrostatic potentials of the set of points defining the van der Waals surface for each molecule; (2) place the transition state and the test molecule at the origin of the coordinate system; (3) keeping the transition state surface fixed, rotate the test molecule until S_g is maximized; (4) score directly the similarity with S_e; and (5) or, alternatively, transform the geometrical surface of the test molecule into that of the transition state and then score with S_e.

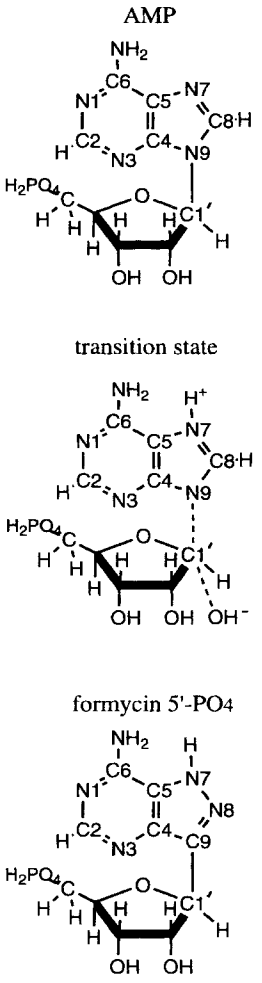

FIGURE 2. Substrate, transition state, and inhibitor for AMP nucleosidase reaction.

Results

A molecular electrostatic potential surface can be visualized directly by coupling the full output of *Gaussian* calculations to the *AVS* program. Color representations of the relevant molecules can be found in [4, 14, 15]. These surfaces are faithfully

reproduced by extraction from the *Gaussian* outputs a set of points on the 0.002 electrons/bohr^3 isosurface. A pictorial comparison of the results of these two procedures is offered in [4]. For each point, the Cartesian coordinates and corresponding electrostatic potential are obtained by the second scheme. As mentioned above, 17 points per atom leads to good surface coverage.

First, we consider the similarity of AMP, of (*R*)-coformycin 5′-PO_4, and of (*S*)-coformycin 5′-PO_4 to the transition state of the AMP deaminase reaction. The surfaces are first compared directly, without any mappings to simpler structures. The results of such a procedure are shown in Figure 3 where the label on each curve indicates the molecule being compared with the transition state. (Note that the top three curves are for the AMP deaminase series, while the bottom two represent AMP nucleosidase.) α is chosen in the range 0.1–0.5 bohr^{-2}. For all values of α in this range (and, indeed, for a much larger spread), the relative orientation of the two surfaces achieved by maximization of S_g is nearly identical and corresponds to the best geometrical overlap. This result is ascertained by visualization of the oriented surfaces. For α = 0.1 bohr^{-2}, a point on one molecule interacts with points on the other up to 1.67 Å away (where the exponential becomes e^{-1}); for the maximum value of α, the search is limited to the 0.75 Å neighborhood, i.e., the interaction length is chosen to be in the vicinity of one bond length, a physically reasonable demand. It is seen from the figure that, for all values of α, (*R*)-coformycin is the most similar to the transition state, with (*S*)-coformycin only slightly more similar than is AMP. The similarity scoring is calculated through S_e. The similarity rankings correlate with the binding constants of these molecules to the enzyme: (*R*)-coformycin is the most similar to the transition state and is the most tightly bound, and so on.

If the geometrical surfaces of AMP, (*R*)-coformycin, and (*S*)-coformycin are mapped to that of the transition state prior to scoring, the similarity ranking is preserved. For α = 0.3 bohr^{-2}, e.g., S_e has values of 0.78, 0.68, and 0.50 for the (*R*)-inhibitor, the (*S*)-inhibitor, and the substrate, respectively, when compared with the transition state.

Results for the comparison of AMP and of formycin 5′-PO_4 to the transition state for the AMP nucleosidase reaction are shown in Figure 3 as well (bottom two curves). Again, we see for these unmanipulated surfaces that the inhibitor is more similar to the transition state than is the substrate

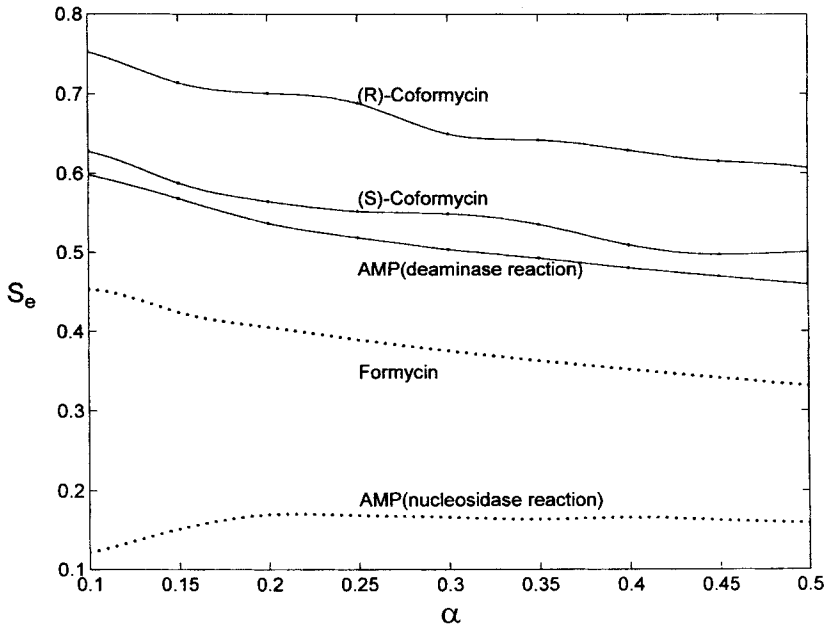

FIGURE 3. Similarity of (*R*)-coformycin 5′-PO_4, (S)-coformycin 5′-PO_4, and AMP with the transition state for AMP deaminase (top three curves). Bottom two curves are similarity measures for formycin 5′-PO_4 and AMP with the transition state for AMP nucleosidase. Each transition state compared with itself has S_e = 1 for all α.

—accordingly, the inhibitor is more tightly bound. Upon mapping of the test surfaces to the transition-state surface, we find for $\alpha = 0.3$ bohr^{-2} that S_e is 0.55 for the inhibitor and only 0.21 for the substrate.

Discussion

Because the organic synthesis of any putative enzyme inhibitor is a time-intensive process, it is most useful to have available beforehand a predication of the inhibitor's efficacy. The scheme that we have offered above allows for such a prediction. As more transition-state structures become available through experiment, inhibitor design for the corresponding enzymatic reactions can thus be facilitated.

Because we use information from the entire molecular electrostatic potential of a molecule without any deformation of the surface, our similarity measure provides for a good comparison of two molecules. Furthermore, by allowing a region on one molecule to explore with exponential decay its similarity to a neighborhood on the other, our scheme takes into consideration that the enzymatic cleft is not a static, rigid structure: rather, an enzymatic residue has the flexibility to accommodate interactions with inhibitor surfaces. The exponential term in the similarity measure serves another function as well. The surface points obtained from the *Guassian* matrices do not give uniform coverage on the van der Waals surface and the exponential "smooths" out such irregularities.

Within the AMP deaminase or the AMP nucleosidase series, all the molecular species are geometrically similar. This allows for an initial geometrical alignment with S_g. However, if two molecules to be compared are less similar in their surface structures, it would be desirable to align them, prior to scoring with S_e, with a *combination* of S_g and S_e.

Finally, the surface-transformation procedure discussed above maps the electrostatic potential distributions of the molecules to the same geometrical surface. We are using the reduced data of these transformed surfaces as input into a neural network to predict numerically the binding constant of a proposed inhibitor.

ACKNOWLEDGMENTS

This work was supported by NIH Grant GM 41916, funds from the Mather Foundation, and from the U.S. Army Medical Research and Material Command.

References

1. R. Wolfenden, Acc. Chem. Res. **5**, 10 (1972).
2. (a) V. L. Schramm, B. A. Horenstein, and P. C. Kline, J. Biol. Chem. **269**, 18259 (1994); (b) V. L. Schramm, B. A. Horenstein, C. K. Bagdassarian, S. D. Schwartz, P. Berti, K. A. Rising, J. Scheuring, P. C. Kline, D. W. Parkin, and D. J. Merkler, Int. J. Quantum Chem.: Quant. Biol. Symp. **23**, 81 (1996).
3. (a) D. J. Merkler, P. C. Kline, P. Weiss, and V. L. Schramm, Biochemistry **32**, 12993 (1993); (b) F. Mentch, D. W. Parkin, and V. L. Schramm, Biochemistry **26**, 921 (1987); (c) D. W. Parkin, F. Mentch, G. A. Banks, B. A. Horenstein, and V. L. Schramm, Biochemistry **30**, 4586 (1991).
4. C. K. Bagdassarian, V. L. Schramm, and S. D. Schwartz, submitted.
5. P. Sjoberg and P. Politzer, J. Phys. Chem. **94**, 3959 (1990).
6. P. Politzer and D. G. Truhlar, *Chemical Applications of Atomic and Molecular Electrostatic Potentials* (Plenum, New York, 1981).
7. P. K. Weiner, R. Langridge, J. M. Blaney, R. Schaefer, and P. A. Kollman, Proc. Natl. Acad. Sci. U.S.A. **79**, 3754 (1982).
8. J. Novotny and K. Sharp, Prog. Biophys. Mol. Biol. **58**, 203 (1992).
9. (a) P.-L. Chau and P. M. Dean, J. Mol. Graph. **5**, 97 (1987); (b) P. M. Dean and P.-L. Chau, J. Mol. Graph. **5**, 152 (1987).
10. M. Wagener, J. Sadowski, and J. Gasteiger, J. Am. Chem. Soc. **117**, 7769 (1995).
11. P. G. Mezey, *Shape in Chemistry: An Introduction to Molecular Shape and Topology* (VCH, New York, 1993).
12. (a) G. Tasi and I. Polinko, Top. Curr. Chem. **174**, 45 (1995); (b) R. Carbó, L. Leyda, and M. Arnau, Int. J. Quantum Chem. **17**, 1185 (1980); (c) W. G. Richards and E. E. Hodgkin, Chem. Br. **24**, 1141 (1988).
13. (a) S. K. Kearsley and G. M. Smith, Tetrahedron Comp. Method. **3**, 615 (1990); (b) G. Klebe, T. Mietzner, and F. Weber, J. Comp.-Aided Mol. Des. **8**, 751 (1994).
14. P. C. Kline and V. L. Schramm, J. Biol. Chem. **269**, 22385 (1994).
15. J. I. Ehrlich and V. L. Schramm, Biochemistry **33**, 8890 (1994).