Estimating and representing hydrophobicity potential

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The proximity effects observed in calculating octanol/water partition coefficients of bisubstituted aliphatic chains are interpreted as a measure of the hydrophobicity potential of the substituents. These effects and the derived potentials decay exponentially from the center of the molecular fragment (substituent). A hydrophobicity potential is defined that is proportional to the hydrophobic fragmental constant and has its maximal value in the center of the fragment. Summing up the fragmental hydrophobic contributions enables us to assign a potential to any point in space around the molecule. Selected colored representations of the potential, such as those shown here for enalapril, add to the available pictures of bioactive molecules and should be useful for design purposes in molecular pharmacology.

Keywords: hydrophobicity, lipophilicity, hydrophobicity potential, partition coefficients, enalapril, molecular graphics of hydrophobicity

Received 9 February 1988
Accepted 21 March 1988

The interaction of a bioactive molecule with its receptor is controlled not only by the spatial complementarity of their three-dimensional (3D) structure and the electrical complementarity of their charges at the surface, but also by the hydrophobic interactions between their contact surfaces. Merely representing the van der Waals volume even in the proper molecular conformation and the electrical potential of the effector molecule is therefore inadequate and should be complemented by an appropriate description of the hydrophobicity around the molecule.

It is reasonable to expect a solute molecule to exert forces on its environment (solvent or other solute molecules) that originate in its hydrophobicity (lipophilicity) potential.[1] Due to this potential, vicinal molecules orient in a nonhomogeneous way, while a hydrophobic solute tends to attract lipophilic neighbors and repulse hydrophilic ones. This so-called hydrophobic effect[2] results from the tendency of water to exclude hydrophobic groups and of the latter to associate in such a way as to present a minimal surface to water. The corresponding statement is true for a polar molecule in an organic solvent. The orienting effect due to the hydrophobic potential decreases with increasing distance from the solute and is no longer noticeable at sufficiently high separation.

Like any other potential, the hydrophobicity potential has a defined value at each point in space around the molecule. However, to our knowledge, no clear definition or representation of this hydrophobicity potential exists in the literature. The purpose of this work was therefore to estimate the value of the hydrophobicity potential for any molecule or molecule fragment; to establish the distance-dependent decay and the range of the potential around the molecule or molecule fragment; and to obtain a 3D representation of this potential in molecular graphics.

METHODS

We assumed that the hydrophobicity potential could be derived from the free-enthalpy change due to the solute transfer from *n*-octanol to water, and we used partitioning data in this system[3,4] as the fundamental experimental values.

Initially bifunctional molecules of the types X-$(CH_2)_n$-X or X-$(CH_2)_n$-Y in which X and Y are relatively polar groups attached via an aliphatic spacer, $(CH_2)_n$, of variable length, were used to estimate the range and decay of the hydrophobicity potential. Proximity effects observed by Rekker[3] and by Hansch and Leo[4] when calculating the partition coefficients for these molecule types were interpreted as the result of the hydrophobic (hydrophilic) interaction of X and Y and therefore as a measure of their hydrophobic potential. The functional dependence of the proximity effects on the distance between the groups X and Y was established by curve fitting using classical statistical tests for evaluating tightness of the fit.

For the molecular graphics, we used a program developed in the IBM Scientific Center of Winchester, UK. We adapted the software to fit the needs of representing the steric features of molecules (van der Waals volume) and the hydrophobic potential in any point

near the molecule. Our program went through four steps, as follows:

(1) Compute and draw the van der Waals surface of the molecule using as input values the coordinates and the radii of the constitutive atoms. Assigning colors to individual atoms led to a molecular picture that was analogous to the well-known CPK models.[5]

(2) Compute the values of the hydrophobicity potential Φ for each point in a cube containing the molecule, by summing up the contributions to Φ from each fragment. When enalapril was represented, the number of points was 134 x 10^6 and the dimensions of the cube were 20 x 20 x 20 (A°).[3] Input values were the fragment potentials Φ_i calculated by equation 5.

(3) Assign a wave length from the visible spectrum to any Φ-value, whereby red represents the lowest field values (strongly hydrophilic) and blue the highest ones (hydrophobic).

(4) Draw the potential Φ in one of the possible representations, such as painting the van der Waals surface with the color corresponding to the local hydrophobicity, drawing colored equipotential surfaces with a selected Φ-value or drawing colored cross-sections of the molecule — centered, for example, on a given fragment.

We performed the calculations on a IBM 4341 mainframe and displayed the images on an IBM 5080 graphic system.

RESULTS

Definition and properties of the hydrophobicity potential

The partition coefficient P in the model system n-octanol/water is a widely accepted way to express quantitatively the hydrophobicity of a molecule. According to the empirical rules derived by Rekker[3] and Hansch and Leo,[4] the partition coefficient P of molecules $X-(CH_2)_n-Y$ (or $X-(CH_2)_n-X$) in octanol/water is given by the equation:

$$\log P - f(CH_2)_n = (f_X + f_Y)(1 - F_P),\ n = 1, 2, 3 \ldots \quad (1)$$

in which fs are the fragmental hydrophobicity constants and F_P the correcting factor for proximity of the groups X and Y. The fragmental contribution $f(CH_2)_n$ of the aliphatic spacer is known with high accuracy and can be treated separately as done in Equation 1. The terms in Equation 1 can easily be transformed to represent the free enthalpy of transfer $\Delta G°$ of the molecule from water to octanol:

$$\Delta G° = 2.3\ RT \log P = 2.3\ RT\,[f(CH_2)_n + f_X (1 - F_P) + f_Y(1 - F_P)] \quad (2)$$

in which R is the gas constant and T the absolute temperature.

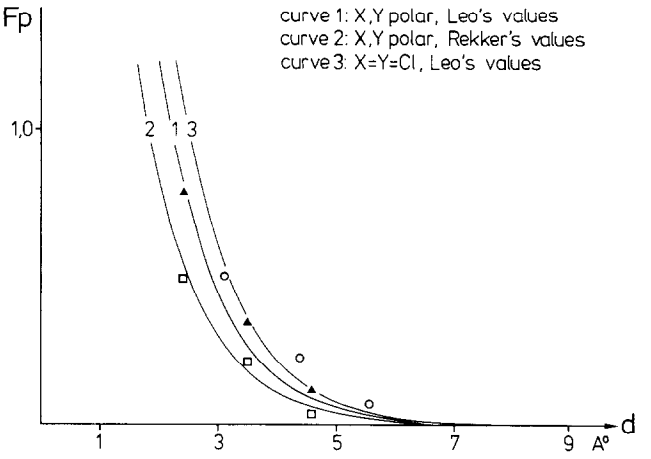

Figure 1. Curve fitting by exponential functions $c \times e^{-ad}$ *($a = 1$, c = empirical constant) of the proximity effects* F_P *(c.f., equation 1). The values fitted are Leo's* F_Ps[4] *in curve 1, Rekker's proximity corrections*[3] *in curve 2, both for polar groups, and Leo's* F_P *values for* $Cl-(CH_2)_n-Cl$ *molecules, when expressed as in equation 1*

The nature of the proximity effects represented by F_P is not clearly understood. However, since they are seen as changes in hydrophobicity, we reasoned that they could be used to estimate the hydrophobicity potential of X and Y. For sufficiently large distances d, the groups X and Y (and the spacer group $(CH_2)_n$) behave independently and no correcting factor appears.

$$\lim\ (d \to \infty)\ F_P = 0 \quad (3)$$

We concluded that for large d values, the contributions of X and Y to $\Delta G°$ were then approximately the same in the molecule as they would be for the isolated fragments. When X and Y get close (i.e., for $n \leqq 3$), they interfere in such a way that the overall hydrophobicity of the molecule is noticeably increased. This experimentally accessible effect provided the basis for the estimation of the range and magnitude of the hydrophobicity potential of X and Y.

The quantity $2.3\ RT\ (f_X + f_Y)\ F_P$ appears as an interaction potential between X and Y that depends on the distance d between the two groups. Although only discrete values of F_P were available, corresponding to a separation of X and Y by one, two or three carbon atoms, we postulated that they could be described by a continuous function $g(d)$ of the distance d between X and Y. For common polar groups, such as OH, NH2, COOH, and so on, the same empirical values of F_P are observed,[3,4] and we found that they were tightly fitted by the exponential function:

$$g(d) = F_P(d) = ce^{-ad} \quad (4)$$

in which c and a are empirical constants. In addition, when d was expressed in Angströms, a was not significantly different from unity (Figure 1).

Extending our investigations to the dihalogenated molecules $Cl-(CH_2)_n-Cl$, for which the fragmental constant is positive in contrast to polar X and Y, we

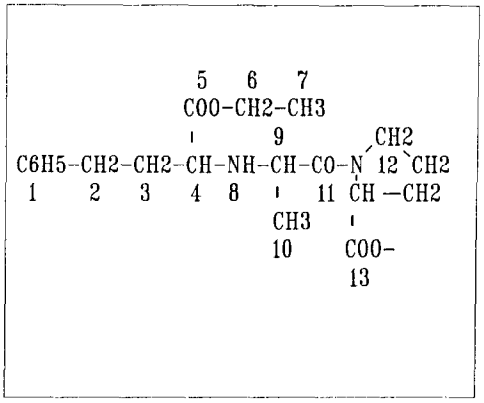

Figure 2. Dividing enalapril into 13 fragments

observed that the proximity effect $-F_p$ also exponentially decayed according to Equation 4. We therefore defined the hydrophobicity potential Φ of X and Y:

$$\Phi_X = 2.3\ RTf_X\, e^{-d},\ \Phi_Y = 2.3\ RTf_Y\, e^{-d} \quad (5)$$

The potential had the expected property:

$$\lim\ (d \rightarrow \infty)\ \Phi = 0 \quad (6)$$

and its maximal value was in the center of the fragment. It exponentially decayed toward zero. The range where it took significant values (i.e., values that would be equal or greater than the experimental error in the measurement of log P) could be estimated easily from Figure 1. This range was situated between 7 and 10 Å, depending on the absolute value of f for the given fragment.

REPRESENTING THE HYDROPHOBICITY POTENTIAL

We tested our method on the well-known angiotensin converting enzyme inhibitor enalapril,[6] which was tentatively divided into 13 fragments (Figure 2), including 9 different fragment types. A center was assigned to each fragment, namely, for fragment 1, the center of the aromatic ring; for fragments 2, 3, 4, 6, 7, 9, 10, the center of the carbon atom; for fragments 5 and 13, the center of the C–O–O triangle; for fragment 11, the middle of the C=O bond; for fragment 8, the center of the nitrogen atom; and for fragment 12, the center of the pyrrolidine ring. Values of the hydrophobicity potential were represented only outside the van der Waals volume. The highest absolute values of the potential were $f_i \times e^{-d}$min, in which f_i was the fragment constant[3,4] and d_{min} the radius of the smallest van der Waals sphere containing the fragment. Table 1 shows those values and those at larger distances d for each fragment type.

The results of the summations of these contributions to the true hydrophobicity potential in each point of the surface or in the vicinity of the molecule are represented graphically in Color Plates 1 to 4, and their respective legends explain the meaning and the merits of each representation. Especially informative is the picture of the equipotential surfaces in Color Plate 3, which directly reflects the hydrophobic features of the (enalapril) receptor surface. The display of the pictures can be varied *ad libitum*. Hence, observation point, position of the cross-section or value of the potential to be displayed in the contours are optional.

Color Plate 5 also shows the electrostatic potential around the enalprilat molecule. This representation, although not directly comparable to Color Plate 4, clearly shows that hydrophobic and electrostatic potentials display very different features — for example, in respect to the spatial location of their maxima and minima.

DISCUSSION

The graphic representation of the hydrophobicity in the vicinity of an effector molecule is a necessary complement to the picture of its steric bulk and its electric field. In general, hydrophobic and electric fields behave in very different ways, since, for example, an ammonium group and a carboxylate group display similar hydrophobic but opposite electric potentials.

The hydrophobic field defined in this work relies upon experimental values of the partition coefficient and empirical rules for its calculation. Although we could take into account only a small number of groups X and Y, generalizing about the proximity effects seemed justified, since we investigated both groups with positive and negative fragmental constants. In contrast to the hydrophobicity potential proposed by Aubry *et al.*,[1] for which only the boundary conditions could be checked, the hydrophobicity potential defined here and its observed exponential decay rest on direct experimental evidence. The definition of the potential range as being the distance d up to which values of the proximity effect significantly affects the calculated partition coefficients should also be reasonable.

In the described procedure, summation over the individual fragment potentials results in a known value for any point in a box containing the molecule to be analyzed. The necessary input for such a calculation is the availability of the atomic coordinates, which may be a general problem. However, for relatively small effector molecules, the picture of the hydrophobicity even in the improper conformation can still reveal fundamental features. Another important step in the procedure is the subdivision of the molecule into fragments, which is made arbitrarily. However, we were led by common sense and by the nature of the groups for which the fragmental constants are tabulated.[3,4] Reducing the molecule into a sum of atoms is generally not appropriate, since the corresponding f' values are not available. The choice of the center of the fragment is also not always trivial, although another possible choice will generally modify the overall picture slightly.

Molecular modeling using computer graphics often exaggerates the role of the steric features in the drug-receptor interaction. The graphic representation described here should serve as an additional tool for designing potent and selective drugs, since it allows researchers to compare the surrounding hydrophobicity

Table 1. Fragment types, fragment constants f, and hydrophobicity potential (divided by 2.3 RT) of the fragments of enalapril at different distances $d_{min} \leqq d \leqq 7$Å from the center of the fragment

Fragment type	f	d_{min}[a])	fe^{-d}min	fe^{-3}	fe^{-5}	fe^{-7}
C_6H_5-	+1.90	3.52	+0.0562	+0.0946	+0.0128	+0.0017
$-CH_2-$	+0.66	1.77	+0.1124	+0.0329	+0.0044	+0.0006
$-CH-$	+0.43	1.77	+0.0732	+0.0214	+0.0029	+0.0004
$-COO-$	−1.49	2.02	−0.1977	−0.0742	−0.0100	−0.0014
$-CH_3$	+0.90	1.77	+0.1533	+0.0448	+0.0061	+0.0008
$-NH-$	−2.15	1.70	−0.3928	−0.1070	−0.0145	−0.0020
$-CO-$	−1.90	2.02	−0.2520	−0.0946	−0.0128	−0.0017
$cNCH(CH_2)_3$	+0.72	2.40	+0.0653	+0.0358	+0.0049	+0.0007
$-COO^-$	−5.19	2.02	−0.6885	−0.2584	−0.0350	−0.0047

[a] d_{min} = radius of the smallest van der Waals sphere containing the given fragment

of series of analogues in relation to the biological activity.

ACKNOWLEDGEMENT

This work was supported by the Swiss National Science Foundation (grant 3.205−0.85). We thank Mr. R. Phippen for excellent assistance.

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