Further Development of Hydrogen Bond Functions for Use in Determining Energetically Favorable Binding Sites on Molecules of Known Structure. 1. Ligand Probe Groups with the Ability To Form Two Hydrogen Bonds

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The directional properties of hydrogen bonds play a major role in determining the specificity of intermolecular interactions. An energy function which takes explicit account of these properties has been developed for use in the determination of energetically favorable ligand binding sites on molecules of known structure by the GRID method (Goodford, P. J. *J. Med. Chem.* **1985**, *28*, 849. Boobbyer, D. N. A.; Goodford, P. J.; McWhinnie, P. M.; Wade, R. C. *J. Med. Chem.* **1989**, *32*, 1083). In this method, the interaction energy between a target molecule and a small chemical group (a probe), which may be part of a larger ligand, was calculated using an energy function consisting of Lennard-Jones, electrostatic, and hydrogen bond terms. The latter term was a function of the length of the hydrogen bond, its orientation at the hydrogen-bonding atoms, and their chemical nature. We now describe hydrogen bond energy functions which take account of the spatial distribution of the hydrogen bonds made by probes with the ability to form two hydrogen bonds. These functions were designed so as to model the experimentally observed angular dependence of the hydrogen bonds. We also describe the procedure to locate the position and orientation of the probe at which the interaction energy is optimized. The use of this procedure is demonstrated by examples of biological and pharmacological interest which show that it can produce results that are consistent with other theoretical approaches and with experimental observations.

Introduction

The ability to determine ligand binding sites on biological macromolecules is of importance in the design of therapeutic agents.[1,2] A procedure, known as the GRID method has been developed for determining energetically favorable binding sites for small chemical groups (probes) on a target molecule or group of molecules.[3-5] The target may be a macromolecule, e.g. a protein, nucleic acid, or polysaccharide, or a small molecule, e.g. a drug, or any combination of such components. The probe groups are small, distinct chemical entities such as a carbonyl oxygen or an amine nitrogen, which may be constituents of a larger ligand, such as a drug.

In the GRID method, the probe group is moved through a regular grid of points in a region of interest around the target molecule and, at each point, the interaction energy between the probe and the target molecule is calculated using a classical empirical energy function. The energies calculated at these points may be displayed as three-dimensional contours around the target molecule using computer graphics. Contours at large negative energies indicate energetically favorable binding regions for the particular probe while those at large positive energies correspond to regions from which it would be repelled. During each GRID calculation, the target molecule is held stationary although the mobility of its hydrogen atoms and lone pairs of electrons is taken into account. Therefore, each GRID map describes the properties of one particular conformation of the non-hydrogen atoms of the target molecule, and a number of GRID maps can then be generated for different conformations if this is appropriate.

Hydrogen bonds play a crucial role in determining the specificity of ligand–macromolecule interactions.[6,7] This specificity arises because hydrogen bonds are short-range interactions dependent on the chemical nature of the hydrogen-bonding atoms, and are directional, unlike dispersive and electrostatic point charge interactions which are isotropic. These properties are due to the complex nature of the hydrogen bond to which electrostatic, charge-transfer, polarization, dispersion, and electron-exchange terms contribute (see ref 4 and references therein). The attractive interaction appears to be largely due to the electrostatic[8] and charge-transfer[9,10] components. Therefore, for the determination of ligand–macromolecule interactions, the GRID method uses an energy function which contains an explicit hydrogen bond term (E_{hb}) in addition to Lennard-Jones (E_{lj}) and electrostatic (E_{el}) terms. The total energy (E) is the sum of the pairwise interactions between the probe and all the atoms of the

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(3) Goodford, P. J. A Computational Procedure for Determining Energetically Favorable Binding Sites on Biologically Important Macromolecules. *J. Med. Chem.* **1985**, *28*, 849-857.

(4) Boobbyer, D. N. A.; Goodford, P. J.; McWhinnie, P. M.; Wade, R. C. New Hydrogen-Bond Potentials for Use in Determining Energetically Favorable Binding Sites on Molecules of Known Structure. *J. Med. Chem.* **1989**, *32*, 1083-1094.

(5) This procedure is implemented by program GRID and inquiries regarding its availability should be directed to the authors.

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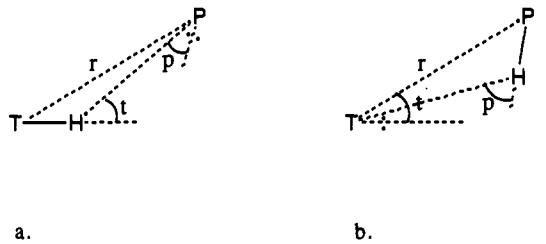

Figure 1. The definition of angles t and p when a hydrogen bond is (a) accepted by and (b) donated by the probe P. T represents a target atom. The orientation of the postulated lone pair electrons on the probe or the target atom is represented by the dots. (The probe also makes another hydrogen bond (not shown) which is stronger than the nonlinear hydrogen bond illustrated here and for which $p = 0°$.)

target molecule:[3]

$$E = \sum E_{lj} + \sum E_{el} + \sum E_{hb} \quad (1)$$

The E_{lj} and E_{el} terms have been described in previous papers.[3,4] The hydrogen bond function, E_{hb}, is given by

$$E_{hb} = Er \times Et \times Ep \quad \text{for } 0 < Et < 1 \text{ and } 0 < Ep < 1 \quad (2)$$

where Er is a function of the separation r of the hydrogen-bonding atoms given in kilocalories/mole, and Et and Ep are dimensionless functions of the angles t and p made by the hydrogen bond at the target and probe atoms respectively (see Figure 1). Hydrogen atoms and lone-pair electrons are not represented explicitly as distinct components of the probe. The probe is, instead, described as a sphere with appropriate parameters which take into account the presence of the hydrogen atoms and lone-pair electrons. A similar "extended atom" representation may be used for the atoms of the target or, alternatively, an "all-atom" representation may be used. In both the extended form and the all-atom models, the positions of polar hydrogen atoms in the target are calculated in order to evaluate the hydrogen-bond energy. In addition, in the all-atom model the positions of all other hydrogen atoms are also calculated. The lone pairs of electrons and the hydrogen atoms shown in Figure 1 are used to calculate the hydrogen bond energy. In the extended atom representation, they do not contribute to E_{lj} or E_{el}. In the all-atom representation of the target, E_{lj} and E_{el} are calculated for the hydrogen atoms but not for the lone pairs of electrons.

The empirical energy functions used in molecular mechanics calculations may be separated (see references 4 and 11 and citations therein) into those, like the GRID method, that contain an explicit hydrogen-bond term, e.g. refs 12, 13, and 14, and those that do not, e.g. refs 15 and 16. In the alternative approach to calculating the contribution of hydrogen bonds in which an explicit hydrogen bond term is not used in the energy function an all-atom representation is usually necessary. This approach is not normally used in the GRID method for several reasons: (1) The use of functions Et and Ep allows for the mobility of the hydrogen atoms and lone pairs of electrons which would not be possible if they were assigned rigid coordinates. Examples of mobile hydrogen atoms and lone pairs of electrons include those in the hydroxyl group of tyrosine residues and in the tautomers of histidine residues.[4] (2) The extended atom representation is less computationally intensive than the all-atom representation. (3) Without the explicit lone-pair orbitals, the directional properties of accepted hydrogen bonds are not easily modeled in the all-atom representation. This is of particular importance for the probe group because its covalently attached atoms, which affect hydrogen bond geometries through steric effects, are not included in the calculations.

In previous versions of the GRID programs,[3,4] $Ep = 1$ was always used. This meant that, at some grid points, two strong hydrogen bonds could be computed to subtend an unrealistically small angle at the probe, and their deviation from linearity would not be considered in calculating their energy. The new version of the GRID program described in this paper computes Ep and, hence, the hydrogen bond energy, with due consideration of the distortion of the hydrogen bonds from their optimum geometry at the probe, and thus prevents the simultaneous formation of two hydrogen bonds which subtend a very acute angle at the probe. This results in the location of energetically favorable binding regions for the probe which tend to be smaller and better defined than previously.

In this paper, Ep is described in detail; Er and Et have been described earlier[4] and are, therefore, only outlined briefly here. Er, Et, and Ep have been modeled so as to reproduce the geometries of experimentally observed hydrogen bonds in small and large molecules. The distance dependence, Er, of the hydrogen bond energy is given by:

$$Er = C/r^8 - D/r^6 \quad (3)$$

where C and D are dependent on the chemical nature of the interacting atoms.[4] It is evaluated when r is less than a cutoff distance, typically 5 Å.

For target atoms that donate hydrogen bonds, Et is given by:

$$Et = \cos^n t$$

$n = 2$, 4, or 6 according to the type of atom[4] (4)

When the donated hydrogen atom of the target can occupy more than one position, Et is calculated for the most favorable position of the donated hydrogen atom. For example, the hydrogen of an sp^3-hybridized hydroxyl group is located at the position on its circular locus at the tetrahedral angle around the C–O bond at which it can make the strongest hydrogen bond. In histidine residues, the hydrogen is assumed to be bonded to the imidazole nitrogen which results in the formation of the strongest hydrogen bond.[4]

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(13) Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, U. C.; Ghio, C.; Alagona, G.; Profeta, S., Jr.; Weiner, P. A New Force Field for Molecular Mechanical Simulation of Nucleic Acids and Proteins. *J. Am. Chem. Soc.* **1984**, *106*, 765–784.

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For target atoms that accept hydrogen bonds, *Et* takes a variety of functional forms in order to reflect the different observed geometries of accepted hydrogen bonds. The mobility of the lone pairs of electrons of the target atoms is treated in a similar fashion to that of hydrogen atoms.

In the GRID method, the probe is free to rotate at each position at which the interaction energy is calculated because the positions and chemical identities of the atoms covalently bound to it are undefined. The probe therefore has more degrees of freedom than a target atom of the same chemical type, and different methods of calculating the hydrogen bonds formed by a probe and by a target atom are necessary.

Two distinct aspects may be considered in determining the dependence of the strength of hydrogen bonds on their orientation at the probe:

(1) The functional form, *Ep*, of the angular dependence of the individual hydrogen bonds made by the probe. This was modeled to fit experimental data on hydrogen bond geometries in the same way as *Et*.[4]

(2) The determination of the combination of those individual hydrogen bonds which results in the most favorable total probe–target interaction energy *E*. This stage of the computation varies in complexity according to the hydrogen-bonding capacity of both the probe and the target.

If only one hydrogen bond is formed by the probe at a particular grid point, the probe may be assumed to orient itself so that the hydrogen bond is optimally aligned[18] with angle $p = 0°$ and $Ep = 1.0$. It adopts this orientation because all the other forces which act upon it are, to a first approximation, isotropic. Note, in particular, that its orientation is not restricted by covalent bonds to any other atom or group. If the hydrogen bond is donated by the probe, it is assumed to lie in the direction of the probe's hydrogen atom. If the hydrogen bond is accepted by the probe, it is assumed to lie either in the direction of one of the probe's lone-pair orbitals, or along the bisector of the two lone-pair orbitals.

If more than one hydrogen bond is formed by the probe, its environment may be such that not all of its hydrogen bonds can be optimally oriented. The energies of the weaker hydrogen bonds are then multiplied by a fraction Ep ($0 < Ep < 1$), in order to account for their less favorable geometry. In addition, at some positions of the probe on the array of grid points at which the interaction energy is calculated, the hydrogen-bonding capacity of the target or of the probe itself might not be fully satisfied. In these cases, program GRID must determine which hydrogen bonds are formed and which are not, such that the total interaction energy, *E*, is optimized. Thus, the GRID program provides a simplified but rational method of determining the contribution of hydrogen bonds to the strength of the probe–target interaction.

Probes that have the capacity to make only two hydrogen bonds are now considered. The treatment of probes that are able to make three or four hydrogen bonds is described in the following paper.[17]

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Method of Determining the Hydrogen Bonds Made by the Probe

The Derivation of Function *Ep*. Function *Ep* was derived by fitting to experimental data from neutron and X-ray diffraction studies as described previously for the derivation of *Et*.[4] The same experimental data were used as before (Table I in ref 4) except that, for atoms for which separate data were available for the cases when they made one hydrogen bond and when they made more than one hydrogen bond, data was only used for the cases where more than one hydrogen bond was made. This was because *Ep* is only less than 1.0 when the probe is making more than one hydrogen bond and because the hydrogen bond geometry of a particular atom is influenced by the number of hydrogen bonds it makes. For instance, for a carbonyl oxygen atom, it is quite common for a hydrogen bond to occur near the bisector of the lone-pair orbitals if the oxygen accepts only one hydrogen bond. If the carbonyl oxygen atom accepts two hydrogen bonds, however, a hydrogen bond pointing in this direction is unlikely.[19–21] Data on the hydrogen bond geometry of atoms making two hydrogen bonds were available for carbonyl oxygens accepting a hydrogen bond from an amide nitrogen (174 hydrogen bonds)[19] and for oxygens accepting a hydrogen bond from a hydroxyl group (90 hydrogen bonds).[20]

As in the derivation of function *Et*,[4] the experimental distribution of the hydrogen bond geometries was converted to probabilities and then to energies assuming a Boltzmann distribution. These energies were then plotted against angle *p* and an analytical function *Ep* was chosen to fit them. This function was required to be continuous and simple to compute while reproducing experimentally observed hydrogen bond geometries with reasonable accuracy.

The following expression for *Ep* was found to be appropriate for calculating the energy of the weaker hydrogen bond made by probes with the capacity to form two hydrogen bonds:

$$\begin{aligned} Ep &= \cos^2 p \quad 0° < p < 90° \\ &= 0 \quad p > 90° \end{aligned} \tag{5}$$

where *p* is defined in Figure 1. Thus $Ep = 1.0$ when the angle subtended at the probe by the two hydrogen bonds is the ideal angle for that particular probe, and *Ep* is less than 1.0 when the angle subtended at the probe by the two hydrogen bonds deviates from the ideal angle. This is, to a first approximation, either the tetrahedral (109°) or the trigonal (120°) angle. For carbonyl, carboxyl, and phenolate oxygen, phenolate hydroxyl, and sp^2-hybridized nitrogen probes, the ideal angle was taken as 120°, whereas for ether oxygen and sp^3-hybridized nitrogen probes, it was 109°. This function for *Ep* simulates the experimental observation that stronger, shorter hydrogen bonds in general show less deviation from linearity than longer, weaker hydrogen bonds.[18,22,23]

The Treatment of Multiple Hydrogen Bonds. Preliminary calculations for an sp^2-hybridized amide nitrogen

(19) Ramakrishnan, C.; Prasad, N. Study of Hydrogen Bonds in Amino Acids and Peptides. *Int. J. Prot. Res.*, 1971, *III*, 209–229.

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(22) Taylor, R.; Kennard, O.; Versichel, W. The Geometry of the N—H···O—C Hydrogen bond. 3. Hydrogen bond Distances and Angles *Acta Crystallogr.* 1984, *B40*, 280–288.

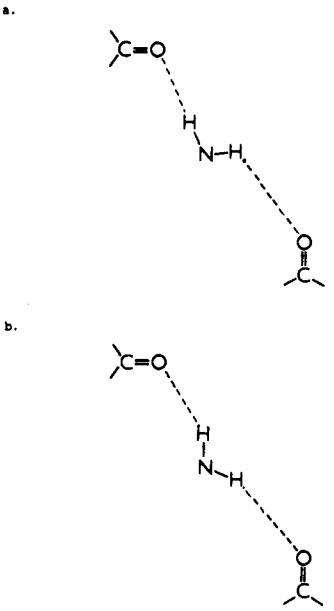

Figure 2. The geometry of the hydrogen bonds donated by an sp^2-hybridized amide nitrogen $-NH_2$ probe to a target containing two carbonyl groups with all atoms shown coplanar for clarity. The target atoms are in fixed positions, and the probe is free to rotate. (a) Asymmetric orientation of the probe with an N–H bond coincident with an N···O vector. (b) Symmetric orientation of the probe with the bisector of angle H–N–H coincident with the bisector of angle O–N–O.

$-NH_2$ probe interacting with two target carbonyl oxygen atoms showed that an asymmetric orientation of the probe (as defined in Figure 2) almost always resulted in a more favorable total hydrogen bond energy than a symmetric one. This is shown by the plots in Figure 3 in which the dependence of the hydrogen bond energy on the position of the target carbonyl oxygens with respect to the probe is given. These plots show that, in general, the asymmetric arrangement results in a more favorable hydrogen bond energy than the symmetric arrangement. Only when two hydrogen bonds of near optimal geometry are formed by the probe does the symmetric arrangement result in a more favorable hydrogen bond energy than the asymmetric arrangement (Figure 3a). In this case, however, the difference in the hydrogen bond energies calculated for these two arrangements may be considered to be negligible. In these calculations, the effect of solvent was "averaged" through the use of a continuum representation so that a general rule regarding the symmetry of multiple hydrogen bonds at a probe could be deduced. However, in specific cases, differential solvation of the hydrogen bonding groups by individual water molecules may result in different energy profiles.

These hydrogen bond energies may be explained by the fact that, in the asymmetric case, *Ep* is less than 1.0 only for the weaker hydrogen bond and the full strength of the stronger hydrogen bond is always retained, whereas in the symmetric case, *Ep* is less than 1.0 for both hydrogen bonds. The results of this calculation are supported by NMR studies[24] which suggests that, for a water molecule hydrogen bonded to a protein, one hydrogen bond is stronger than the others so that the water molecule has

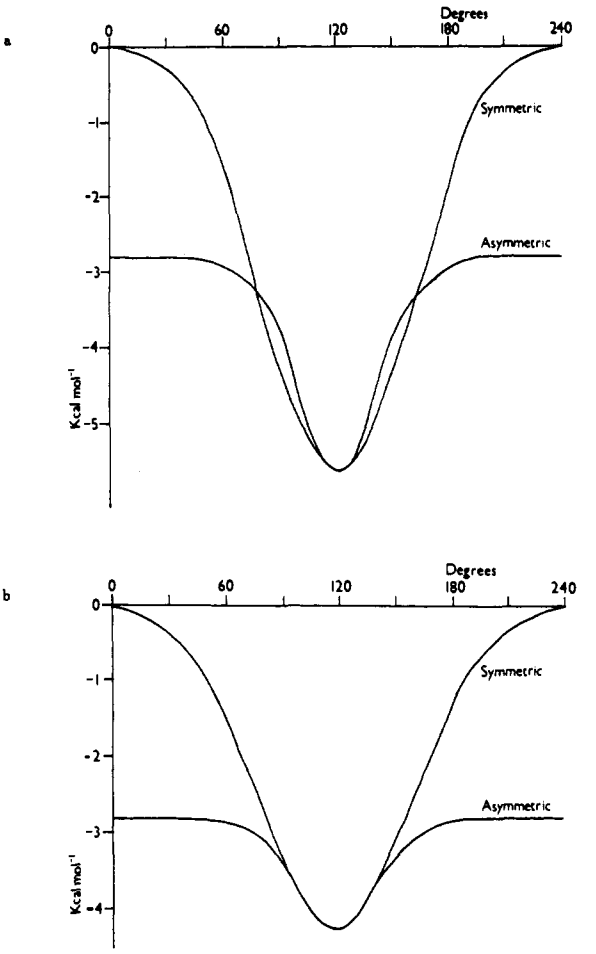

Figure 3. Plots of the hydrogen bond energy, E_{hb} versus angle O–N–O for an sp^2-hybridized amide nitrogen $-NH_2$ probe interacting with two target carbonyl oxygen atoms as illustrated in Figure 2. (a) Both target carbonyl oxygen atoms 3.0 Å away from the probe. (b) One carbonyl oxygen atom at 3.0 Å and one at 4.25 Å from the probe. E_{hb} is almost always more favorable for the asymmetric arrangement of the probe and the target atoms (Figure 2a) than for the symmetric arrangement (Figure 2b).

an asymmetric arrangement of hydrogen bonds and its motion is rotationally anisotropic. Accordingly, in the GRID method, hydrogen bonds are assigned an asymmetric configuration in which the strongest hydrogen bond is aligned optimally at the probe and has $Ep = 1.0$, while the weaker one deviates from linearity at the probe by an angle p and is therefore modified by an appropriate angular factor $Ep < 1.0$.

The determination of which hydrogen bonds are made by the probe is illustrated for a carbonyl oxygen probe by the examples in Figure 4 and Table I. In such a situation, program GRID considers all possible combinations of hydrogen bonds and finds those which result in the most favorable total interaction energy *E*. As a result, the hydrogen bonds that are selected may not always include the hydrogen bond which is calculated to be the strongest on the basis of the individual values of *Er* and *Et* alone (see Table I).

Examples of the Application of the Hydrogen Bond Functions

Noradrenaline. L-Noradrenaline (NA) serves as a suitable target molecule with which to demonstrate the effect on the calculated hydrogen bond energy of taking the hydrogen-bonding geometry of the probe into account.

(23) Olavsson, I.; Jonssen, P. G. *The Hydrogen-Bond*; Schuster, P., Zundel, G., Sandorfy, C., Eds.; North-Holland: Amsterdam, 1976, Volume II, Chapter 8.

(24) Bryant, R. C. Water at the protein surface. In *Biophysics of Water*, Franks, F., Ed.; Wiley: New York, 1981; pp 178–181.

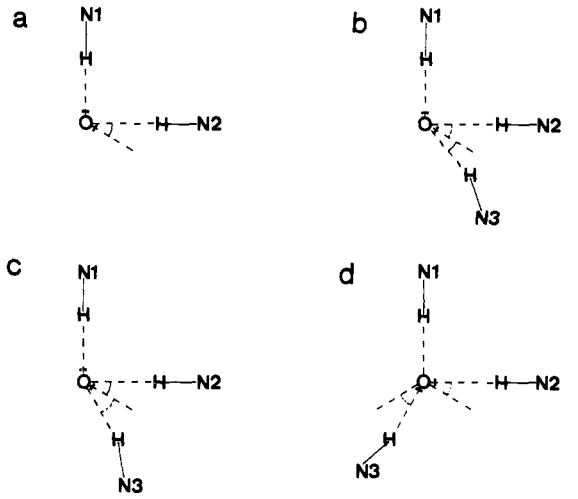

Figure 4. The determination of the hydrogen bonds accepted by a carbonyl oxygen probe from different model target molecules containing nitrogens which are able to donate hydrogen bonds. The hydrogen bonds formed and their computed energies are listed in Table I. The plane of the paper corresponds to the plane of the probe's lone-pair orbitals, which are represented by dots, and all atoms are shown coplanar for clarity. Angle p is shown by a solid line for hydrogen bond predicted to be formed and by a dashed line for those predicted not to be formed. (a) The target contains two hydrogen-bonding atoms only. (b) The target contains three hydrogen-bonding atoms. The hydrogen bond to N3 deviates from linearity at both the probe (p = 20°) and at the target (t = 20°). Despite this, a stronger second hydrogen bond can be formed to N3 than to N2 which is further from the probe and at a less favorable position with respect to the probe's lone pairs of electrons (p = 30° for a hydrogen bond to N2). (c) The target contains three hydrogen-bonding atoms and is the same as in (b) except that the position of N3 is shifted. This shift means that the second hydrogen bond is now made to N2. For both N2 and N3, p = 30°, but the hydrogen bond to N2 is slightly stronger because its N–H bond is aligned with the vector from N2 to the probe. (d) The target contains three hydrogen-bonding atoms and is the same as in (b) and (c) except that the position of N3 is shifted. The hydrogens covalently attached to N2 and N3 subtend an angle of 120° at the probe and so, as shown in Table I, it is more favorable for hydrogen bonds to be formed to both of these atoms rather than to N1 and only one of them.

NA was used previously[4] to demonstrate the form of function *Et*. Calculations have now been performed with program GRID for three different probes, each of which can make two hydrogen bonds. The three probes were a phenolic sp^2-hybridized hydroxyl group, an sp^3-hybridized hydroxyl group, and a carboxyl oxygen atom. Thus, all the probes contained an oxygen atom but each had differing hydrogen bond properties. The NA was in a crystallographically observed conformation.[25]

In Figure 5a, GRID energy contours are shown for the interaction of a phenolic hydroxyl probe with NA. In Figure 5b, energy contours are shown at the same energy level with the same target molecule for an sp^3-hybridized hydroxyl probe with the same small change (–0.1 e). The differences between the contours in Figure 5a and 5b are therefore due solely to the difference in the geometry of the two probes. The contoured regions are smaller for the phenolic hydroxyl probe (for which the optimum angle subtended by the hydrogen bonds at the probe is assumed to be 120°) than for the sp^3-hybridized hydroxyl probe (for which the optimum subtended angle is assumed to be 109°). This is because of the close proximity of the two atoms of the target molecule which make hydrogen bonds simultaneously to the probe, i.e. the two oxygens on the aromatic moiety of the target, or the nitrogen and alkyl hydroxyl oxygen on the ethanolamine moiety. Thus, at a given position of the probe, angle p is larger, and therefore Ep is smaller, for the weaker hydrogen bond to the phenolic hydroxyl probe than for the weaker hydrogen bond to the sp^3-hybridized hydroxyl probe.

It is thought[26-28] that the positively charged nitrogen of noradrenaline may play a dominant role in the binding of NA to the adrenergic receptor by interacting with a negatively charged group on the receptor, such as a phosphate or a carboxylate group. Therefore, the interaction of a negatively charged (–0.58 e) carboxyl oxygen probe with NA was calculated using program GRID. The energy contours are shown in Figure 5c. The largest contour, which surrounds the most energetically favorable binding site, is at a position where the probe can accept hydrogen bonds from the nitrogen and from the alkyl hydroxyl group, and can also make a strong attractive electrostatic interaction with the positively charged nitrogen. This contour may therefore indicate a region where the adrenergic receptor would interact strongly with noradrenaline. The adrenergic receptor has been modeled by formate,[29-31] and the favorable binding region for the carboxyl probe identified by program GRID is consistent with the minimum-energy position of formate found in molecular mechanics[29,30] and quantum mechanics[31] calculations. Recently, the binding site of the β_2-adrenergic receptor has been modeled based on sequence homology.[32,33] These models suggest that NA may interact in the same manner with the carboxylate moiety of Asp 113 of the receptor protein.

These calculations of the interaction of three different probes with noradrenaline demonstrate the impact on molecular interactions of small changes in the hydrogen-bonding properties of the probe.

Ribonuclease T1. Ribonuclease T1 (RNase T1) is a microbial enzyme which cleaves single-stranded RNA in a highly specific manner by breaking the phosphodiester bond at the 3′-side of guanosine.[34,35] Its specificity has made RNase T1 a valuable tool in RNA sequencing and

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Table I. Determination of the Hydrogen Bonds Accepted by a Carbonyl Oxygen Probe When It Interacts with Different Model Targets

figure[a]	target atom	distance *r*, Å	*Er*, kcal/mol	angle *t*, deg	*Et* ($\cos^2 t$)	angle *p*, deg	*Ep* ($\cos^2 p$)	E_{hb}, kcal/mol[b]	ΣE_{hb}, kcal/mol[b]
4a	N1	3.00	−3.00	0	1.00	0	1.00	−3.00	(−3.00)
	N2	3.25	−2.68	0	1.00	30	0.75	−2.01	(−2.01)
									−5.01
4b	N1	3.00	−3.00	0	1.00	0	1.00	−3.00	(−3.00)
	N2	3.25	−2.68	0	1.00	30	0.75	−2.01	
	N3	3.00	−3.00	20	0.88	20	0.88	−2.32	(−2.32)
									−5.32
4c	N1	3.00	−3.00	0	1.00	0	1.00	−3.00	(−3.00)
	N2	3.25	−2.68	0	1.00	30	0.75	−2.01	(−2.01)
	N3	3.00	−3.00	20	0.88	30	0.75	−1.98	
									−5.01
4d	N1	3.00	−3.00	0	1.00	90	0.00	0.00	
	N2	3.25	−2.68	0	1.00	0	1.00	−2.68	(−2.68)
	N3	3.00	−3.00	20	0.88	0	1.00	−2.64	(−2.64)
									−5.32[c]

[a] The carbonyl oxygen probe is shown interacting with different model targets in Figure 4. [b] The energies of the possible hydrogen bonds, prior to the selection of the two most favorable ones that could be made by the carbonyl oxygen probe, are shown in the penultimate column. The two hydrogen bonds predicted to be formed for each model target are shown in brackets in the last column. Their sum, ΣE_{hb}, gives the total hydrogen bond interaction energy between the probe and the target. [c] If the strongest hydrogen bond were to be accepted from N1, ΣE_{hb} would only be the same as for Figure 4c. However, in this particular case, a more favorable hydrogen bond energy can be achieved if the probe accepts hydrogen bonds from N2 and N3 but not from N1.

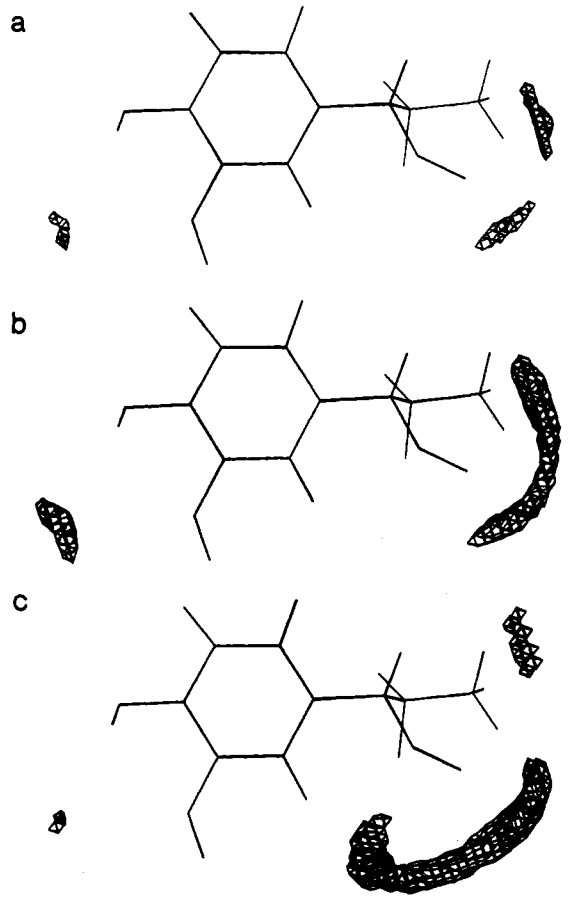


Figure 5. L-Noradrenaline with negative energy contours (at −5.5 kcal/mol) for the interaction with (a) a phenolic sp^2-hybridized hydroxyl probe and (b) an sp^3-hybridized hydroxyl probe. Both probes have a charge of −0.1 e. (c) L-Noradrenaline with negative energy contours for the interaction with a carboxyl oxygen probe with a charge of −0.58 e. See text.

mapping studies. RNase T1 may also provide a useful model for studying the specificity of other nucleotide-binding proteins, many of which are potential targets for the design of therapeutic agents. The high specificity of RNase T1 is not shared by all RNases: other microbial RNases have specificity for adenosine and guanosine, guanosine only, or are nonspecific; the mammalian RNAse A recognizes uracil and cytidine. It is of interest, therefore, to investigate whether program GRID can assist in elucidating how RNase T1 attains its specificity.

A number of crystal structures of RNase T1 have been solved with different inhibitors and ligands.[35] These show that the active site of RNase T1 consists of two parts: a catalytic site and a substrate recognition site. The structure of RNase T1 with the inhibitor 2′-guanylic acid (2′-GMP) has been solved at 1.9-Å resolution with an *R* factor of 18%.[36] Program GRID was run for an amide $-NH_2$ probe (able to donate two hydrogen bonds) in the substrate recognition site of this RNase T1 structure, using Brookhaven Protein Databank[37] file 1RNT with 2′-GMP removed (and with the side chain of Glu 102 modeled in as it was not resolved in the crystal structure). The net atomic charge of the RNase T1 target protein at pH 7 was calculated by program GRID as −9. No counterions were added as the region of most importance for the GRID calculations was the substrate recognition site. Energy maps (see Figure 6) were calculated both with and without considering the distribution of the hydrogen bonds at the probe (i.e. with *Ep* taking up its computationally correct value (Figure 6a) or with *Ep* assigned an arbitary value of 1.0 (Figure 6b)).

Both maps (Figure 6a and 6b) have an energy minimum, corresponding to a favorable binding site for an amide $-NH_2$ group, close to the experimentally observed position of N2 of 2′-GMP. However, Figure 6a shows that the binding region is more precisely defined when *Ep* is calculated correctly, suggesting that the protein receptor site is particularly well designed to exploit the N2 amide group in order to obtain tighter binding of guanine (I) than adenine (II).

The principal difference between the energy maps is shown by the contoured region in Figure 6c. This

(35) Pace, C. N.; Heinemann, U.; Hahn, U.; Saenger, W. Ribonuclease T_1: Structure, Function and Stability *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 343–454.

(36) Arni, R.; Heinemann, U.; Tokuoka, R.; Saenger, W. Three-dimensional Structure of the Ribonuclease-T_1 *2′-GMP Complex at 1.9 Å Resolution. *J. Biol. Chem.* **1988**, *263*, 15358–15368.

(37) Bernstein, F. C.; Koetzle, T. F.; Williams, G. J. B.; Meyer, E. F.; Bryce, M. D.; Rogers, J. R.; Kennard, O.; Shikanouchi, T.; Tasumi, M. The Protein Data Base: a computer-based archival file for macromolecular structures. *J. Mol. Biol.* **1977**, *112*, 535–542.

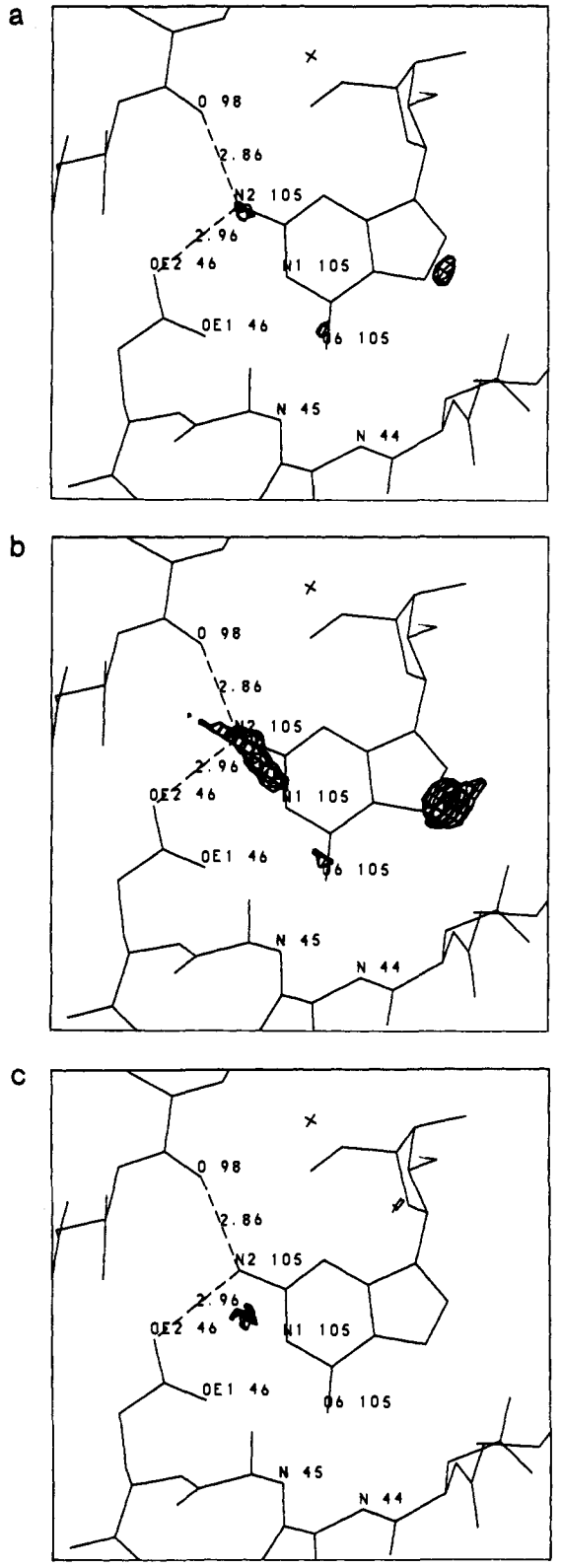
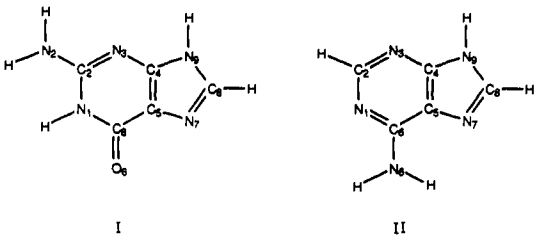


Figure 6. The base recognition site of ribonuclease T1. (a and b) Energy contours (at −8 kcal/mol) indicating favorable binding sites for an amide -NH_2 probe with zero charge are shown (a) with *Ep* calculated to account for the spatial distribution of the hydrogen bonds at the probe, and (b) with *Ep* assigned an arbitrary value of 1.0. (c) Energy map showing the differences between the maps in (a) and (b). The contours show the region where the probe–target interaction is at least 2 kcal/mol less favorable when *Ep* is computed correctly as in Figure 6a than when it is assigned a value of 1.0 as in Figure 6b. See text.

corresponds to a region where, when *Ep* is not calculated, the amide probe is predicted to donate two sizeable hydrogen bonds simultaneously to each of the carboxylate oxygens of Glu 46. However, this is unrealistic because these two hydrogen bonds subtend an angle at the probe in this region that is much smaller than the probe H–N–H angle of 120°. When *Ep* is calculated, this artifact is eliminated and only one hydrogen bond is predicted to be donated to Glu 46.



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Guanine and adenine also differ in their substituents at the 1 and 6 positions. In adenine, O6 is substituted by an amide group and the GRID maps for an amide probe show an attractive region near to O6. This attraction is due to favorable van der Waals interactions and the formation of a hydrogen bond to OE1 Glu 46. Program GRID also predicts an energy minimum near O6 for a carbonyl oxygen probe. The strength of its interaction is predicted to be weaker than for an amide probe due to electrostatic repulsion between the oxygen probe with a charge of −0.3 e and OE1 Glu 46. However, this repulsion is removed when the NH substituent at N1 of guanine is considered as this nitrogen can donate a hydrogen bond to OE1 Glu 46. The presence of the NH group at N1 also prevents the attractive hydrogen bond interaction between OE1 Glu 46 and an amide probe at the O6 position. This shows that N1 and O6 of guanine are positioned to enhance each others capacity to bind to RNase T1. In adenine, N6 might bind favorably to RNase T1 but N1 will not as it cannot donate a hydrogen bond to OE1 Glu 46. This, together with the lack of a binding group at the 2 position, acts against the binding of adenine to RNase T1.

This example shows that the calculation of *Ep* to account for the directionality of hydrogen bonds donated by the probe results in a more precise energy map. It also illustrates the effective use of GRID maps in the interpretion of the ligand binding specificity.

Conclusions

The GRID method is a procedure for locating regions where ligands, represented by probes, bind tightly and selectively to a particular molecular target. Its success depends primarily upon the quality of the GRID energy functions. Here, we have developed the procedure further to improve the modeling of the directional properties of hydrogen bonds. These are of significant importance in determining the specificity of interactions between molecules. Earlier versions of the GRID program took account of the orientation of the hydrogen bonds with respect to the target hydrogen bonding groups. However, the same was not done for the probe which was previously assumed to have spherically isotropic hydrogen bonding properties. We have now modified the GRID procedure to make the calculated hydrogen bond energy depend on the spatial distribution of the hydrogen bonds around the probe and the relative positions of the probe's lone pairs of electrons and hydrogen atoms. This provides a more consistent treatment of the hydrogen-bonding properties of the probe and the target atoms.

The GRID program uses a fast[38] and appropriate rational procedure for determining the total interaction energy between the probe and the target in which the directional properties of hydrogen bonds at both the donor and acceptor atoms (that is both the probe and the target atoms) are considered. It uses an analytic procedure to orient the probe so as to optimize its interaction with the target. A simple function is used to model the dependence of hydrogen bonds on their spatial distribution around the probe. While the current approach ignores some of the more complex features of hydrogen bonds, such as the ability of one donated hydrogen atom to participate in a three-centered hydrogen bond to two acceptor atoms, it does provide a logical and consistent way of calculating hydrogen bond energies. Nevertheless, as more experimental data become available on the geometry of the hydrogen bonds made by donor and acceptor groups which participate in more than one hydrogen bond, it may be appropriate to further modify the present angular functions.

The examples of the use of the new protocol show that it gives more precisely defined ligand binding sites than the approximations used in earlier versions of the GRID program. These show better agreement with experimental observations as exemplified by the improved accuracy of the amide probe binding sites in ribonuclease T1. This means that it is generally easier for the user to identify specific binding sites from the GRID energy maps and to exploit them in drug design. The extension of this approach to probes capable of making three or four hydrogen bonds is given in the following paper.[17]

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(38) The map calculated for an amide nitrogen probe in the substrate recognition site of ribonuclease T1 required less than a minute of central processing time on a Silicon Graphics Indigo workstation.