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Computational analysis of binding affinity and neural response at the L-alanine receptor

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Summary

A model of analogue–receptor binding is developed for the L-alanine receptor in the channel catfish using the AM1-SM2 and ab initio SCRF computational methods. Besides interactions involving the zwitterionic moiety of the amino acid analogue and complementary subsites on the receptor, the model suggests the presence of a hydrophobic pocket with dispersion interactions between the receptor and the residue on the amino acid analogue. Conformational analysis suggests not only a small compact active site on the receptor, but also that the analogues with the highest affinity occupy nearly identical regions of space. Although the binding interaction is dominated by the ionic terms, AM1-SM2 calculations indicate that free energy terms associated with cavity formation, solvent reorganization, and dispersion interactions can be correlated to activation and neural response. From a consideration of this model, molecular features of the analogues that are important for binding and neural response were deduced and other analogues or ligands were developed and tested.

Introduction

Amino acid recognition is an important element of biological systems [1–3]. Bacteria have at least two recognition systems, i.e., the aspartate and serine receptors, whose active sites have been well characterized [2,3]. Amino acid receptors have also been identified in at least two other species, including *Ictalurus punctatus* (the channel catfish) [4a,b] and the Colorado beetle [5]. In each case it is clear that the zwitterionic moiety is the prime element in the interaction between the amino acid analogue and the receptor. The main force is ionic in nature and this interaction is probably the main contribution to binding forces. In the case of bacterial amino acid receptors, the receptor residues surrounding the zwitterionic moieties are an arginine residue interacting with the carboxylate group and a set of residues, including threonine and glutamine, interacting with the positively charged NH_3^+ group [2,3]. Whereas interactions between the receptor and the zwitterionic moiety are common to all amino acid receptors, interactions involving the ligand residue and the receptor are the interactions specific for this class of receptors. For example, in the serine receptor for *E. coli*, the residue interacting with the hydroxyl group on serine is a set of arginine residues, one of which also interacts with the carboxylate group [3]. In the case of the aspartate receptor in *Salmonella typhimurium*, arginine residues on the receptor are involved in the interaction with the carboxylate group of the analogue residue [2]. The role of water in these bacterial systems is quite complicated. In *Salmonella* it has been shown that water plays an integral part in the interactions between aspartate and its receptor [2], whereas clear evidence for receptor–water–ligand interactions is missing in the case of the *E. coli* serine receptor [3].

Amino acid receptors in the catfish have been well characterized by a group of researchers [1,4a,4b,6,7]. Structure–activity data are especially extensive in the case

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of the L-alanine receptor, where a large number of model compounds have been tested and certain features of the ligands or analogues have been determined [4a,b]. The important molecular features for recognition and binding include: (i) the presence of the zwitterionic component in the L-configuration; and (ii) a small residue at the β-carbon (i.e., L-alanine binds to the receptor and activates it, whereas L-phenylalanine does neither). Whereas the interaction of the zwitterionic moiety with the receptor is obviously an ionic interaction, strong in nature and very directional, the interaction between the residue and the receptor, given the nature of the analogues with the highest neural response, is most likely nonpolar in nature. In this regard, an amino acid receptor in the Colorado beetle has been investigated and the stabilizing or activating force between the residue and the receptor is postulated to be hydrophobic in nature [8]. For the L-alanine receptor of the channel catfish, both binding constants and neural response data are available [4a] and the structural features that are important for both binding and activation can be more accurately determined.

In this paper, molecular modeling techniques are used to investigate further the nature of the analogue–receptor binding and activation at the L-alanine receptor in the channel catfish. In general, for many problems in this field, traditional QSAR techniques have been used to correlate the structure of the ligands with activity, circumventing the question of the binding mechanism and the nature of the receptor. In contrast, quantum-mechanical techniques that take into account the presence of solvent can give valuable information with regard to the nature of the binding process as well as the nature of the receptor's active site. This information is of great value when the receptor has neither been isolated nor identified structurally [9].

In the approach presented here, quantum-mechanical techniques are used to determine the low-energy conformations of the amino acid analogues in the specific L-alanine receptor environment. In particular, using both semiempirical AM1 and AM1-SMx [10–13] and ab initio SCRF [14–17] techniques, the low-energy conformations of the best binders can be determined in the receptor environment. By superposition of the accurately determined low-energy conformations, a model of the steric requirements for binding can be formulated. Such a conformational study can, in fact, answer various questions, such as: (i) Do all the analogues have the same conformational profile with respect to the torsion involving the carboxylate group?; (ii) What is the conformational profile around the C^{α}-C^{β} bond in the various analogues?; and (iii) Do all the analogues which have high binding affinities occupy the same regions of space? The answers to these questions can assist in deciphering the steric dimensions of the interaction between the analogues and the receptor. In addition, the placement and orientation of the various zwitterionic and substituent groups can give good insight into the electrostatic requirements of the receptor.

Since the determination of the structure of the particular amino acid analogues using the AM1-SM2 model [10–12] can also generate other important quantities, such as the free energies of cavitation and solvent reorganization and the free energy due to solute–solvent dispersion forces, information regarding the hydrophobic effect can be obtained. Although it is not the dominant contribution to binding, this effect and the resultant dispersion interaction between the residue and the receptor may very well be responsible for the activity of the analogue.

Since all amino acid receptors most likely involve a zwitterionic interaction and frequently include a hydrophobic region on the receptor [2,8], the results of this analysis can be easily extended to other amino acid receptors of interest. These results can also be used to design other ligands of comparable binding affinity, and perhaps to determine whether they would be good activators (agonists) or relatively weak ones (partial agonists or antagonists).

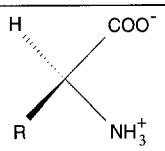
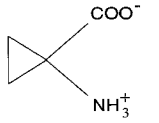
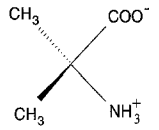
In the next section, the methodology used in the analysis is reviewed. First, the pertinent experimental techniques are briefly described, followed by a discussion of the computational techniques used in the modeling analysis. Subsequently, the computational results for the low-energy conformations in the particular receptor environment are presented and their importance in determining characteristics that are important for analogue–receptor binding is discussed. One possible approach to the analysis of activity is outlined. Finally, conclusions are drawn from the calculations concerning the parameters that are important for binding and activation.

Methodology

Experimental

The experimental techniques, a competitive ligand-binding assay and neurophysiological determination of the biological activity of L-alanine and its analogues, have been described elsewhere [4a]. Briefly, the binding of L-[^{3}H] alanine to a partial membrane preparation (Fraction P2) from taste epithelial membranes was assayed in 0.01 M Tris-HCl, pH 7.8, with 1 mM $CaCl_2$ using a standard ultrafiltration assay [7]. The preparation, handling, and storage requirements for Fraction P2 have been described elsewhere [4a]. Nonspecific binding was determined with unlabeled L-alanine present at 23 mM. Structural analogues of alanine were evaluated as competitors of L-[^{3}H]alanine binding (the total L-alanine concentration was 10^{-6} M) by simultaneous incubation of each analogue and L-[^{3}H]alanine with Fraction P2. The capacity to inhibit ligand binding is reported in terms of IC_{50} values. The values of the IC_{50} were determined from the plot of log X

TABLE 1
CHIRAL AND ACHIRAL SIDE-CHAIN MODIFICATIONS OF L-ALANINE: BINDING INHIBITION AND NEURAL ACTIVITY

No.	Analogue	R	IC_{50} (μM)	Neural response (%)
1		$-CH_3$	2.82 ± 0.64	100
2		$-CH_2Cl$	1.55 ± 0.64	88.6 ± 13.7
3		$-CH_2OH$	3.56 ± 0.88	58.6 ± 15.6
4		$-H$	1.87 ± 0.40	62.3 ± 9.4
5		$-CH_2F$	1.60 ± 0.30	95.7 ± 17.2
6		$-CH_2CN$	33.00 ± 5.00	56.4 ± 14.1
7		$-CH{=}CH_2$	32.00 ± 12.75	77.5 ± 11.3
8		$-CH_2CH_3$	6.53 ± 0.73	59.1 ± 15.1
9		$-CHOH$ $\vert$ CH_3	346.67 ± 61.28	33.5 ± 14.0
10		$-CHCH_3$ $\vert$ CH_3	296.67 ± 77.60	21.1 ± 5.8
11			3.80 ± 0.20	78.3 ± 9.3
12	D-alanine		8.98 ± 3.38	32.0 ± 5.2
13	D-serine		7.05 ± 2.14	–
14			6.88 ± 1.18	35.1 ± 18.7

versus per cent inhibition, where X is the concentration of the ligand or analogue. The concentration of competitor, [X], was varied between 10^{-8} and 10^{-3} M.

The activation of taste receptors by the ligands was measured using standard multiunit recording techniques from the maxillary branch of the facial nerve. Bundles of axons were isolated and the response was determined as the integrated amplitude of the amplified action potentials. Only bundles that responded well to 10^{-6} M L-alanine were used for experiments. The resulting structure–activity relationships and the statistical analyses have been described in a previous publication [4a]. The results for a select group of compounds are given in Table 1. Most of these results have been published earlier; for two compounds (**5** and **10**), the results are from the analysis carried out here.

Computational methods

Computational techniques have been used to rationalize the molecular determinants of binding as well as structural features that may potentially be important for activation. The systems of interest that were studied include all those analogues with an IC_{50} similar to the value for L-alanine (of the order of 1–10 μM). In addition, a few analogues with lower affinities (IC_{50} greater than 10 μM) were also studied. All studies were done for analogues in the zwitterionic state. The computations were undertaken in the following way.

(1) AM1 (or AM1-SM2) semiempirical calculations were carried out in vacuo ($\varepsilon = 1$) and in water ($\varepsilon = 78.5$) for the best binders glycine (**4**), L-alanine (**1**), β-chloro-L-alanine (**2**), β-fluoro-L-alanine (**5**), 1-aminocyclopropane carboxylic acid (**11**) (henceforth referred to as the cyclopropane derivative) and L-serine (**3**). In addition, select calculations were carried out for other analogues including, among others, L-2-amino-3-butenoic acid (**7**) and L-2-aminobutanoic acid (**8**). The SPARTAN 3.1 version of the AM1-SM2 model was used for all semiempirical calculations [18].

AM1 calculations in vacuo were originally done to generate a starting Z-matrix for the geometry optimizations in the ab initio SCRF method (see below), since ab initio calculations on the zwitterion in vacuo were not able to clearly identify any stable conformations. In addition, AM1 calculations in vacuo were done to determine the energy profile of the C^{α}-C[=O] and C^{α}-C^{β} torsions for comparison with the ab initio SCRF results.

AM1-SM2 SCF calculations in water [10–12] were done to generate the free energy term associated with the standard-state free energy of cavity creation in the solvent (a positive term) plus the solute–solvent dispersion forces (a negative term) and a solvent reorganization term. Since it is likely that the neural response depends on the dispersion interactions between the residue and the receptor, AM1-SM2 calculations of this free energy term can be extremely useful in correlating structure with neural response.

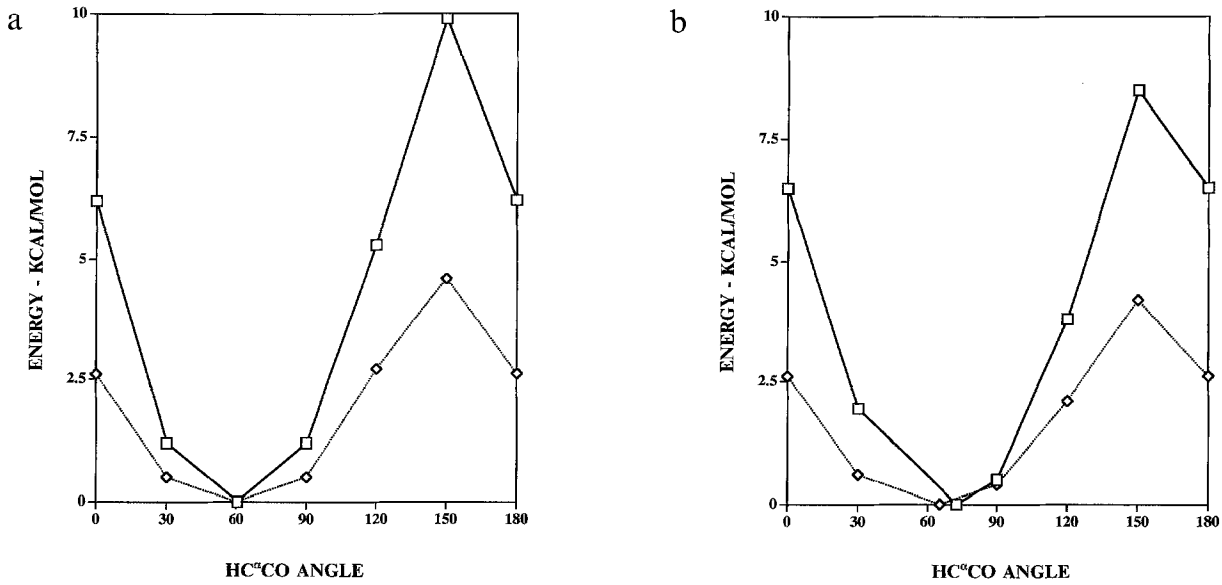


Fig. 1. Comparison of the AM1 (in vacuo) and SCRF-6-31G*//6-31G* ($\varepsilon = 20.7$) relative conformational energies of the (a) glycine and (b) L-alanine zwitterion as a function of the HC$^{\alpha}$CO torsion angle. —□— = ab initio SCRF energy; ···◇··· = AM1 energy.

(2) Ab initio SCRF calculations were performed in order to generate the optimized conformations for the zwitterions in a solvent of moderate polarity (see below). Presently, AM1-SMx calculations can only be performed in hexadecane [13] or in water [10–12]. The ab initio results are fully geometry optimized, the AM1 conformations in vacuo being used as the starting Z-matrix for the ab initio optimizations. For the strong binders, the optimized ab initio SCRF conformational energy profiles for both the C$^{\alpha}$-C[=O] torsion and, where present, the C$^{\alpha}$-C$^{\beta}$ torsion were obtained. The C$^{\alpha}$-N torsion was not studied, as it is highly symmetric and assumed to be relatively low in energy, so that almost all possible conformations are energetically accessible. The torsional studies are especially useful, not only in identifying low-energy conformations but also for comparing conformational profiles for the different systems, i.e., is the low-energy region for the C$^{\alpha}$-C[=O] torsion in all the best binders found in the same torsional angle space?

The ab initio method used was the SCRF formalism of Wong et al. [14–17], available in GAUSSIAN 92 [19]. In this method, the solvent is represented by a continuous medium and the permanent dipole of the solute induces a dipole in the solvent, which in turn interacts with the solute dipole to produce stabilization. Since the multipole expansion of the solute's moments is limited to the dipole term, this significant approximation must be taken into account when evaluating the results for the conformational profiles. However, it might be noted that Wong et al. [15] had some success, using this formalism, in treating the effect of solvent on the structure and energy of the sulfamic acid zwitterion in a moderately polar medium ($\varepsilon = 40.0$). On the other hand, recent work [20] on the conformational energetics of alanine and glycine dipeptides in water ($\varepsilon = 78.5$) seems to indicate the necessity of including higher terms in the multipole expansion.

Crucial to this method is the choice of basis set, solute radius, and dielectric constant. The basis set used was the 6-31G* set. Although diffuse orbitals might be warranted due to the presence of the anionic group, the energy profile for the C$^{\alpha}$-C[=O] torsion in the glycine zwitterion is almost identical for the 6-31G* and 6-31+G* basis sets (not shown). The ab initio results reported here have been obtained with 6-31G*, including fully optimized 6-31G* geometries (6-31G*//6-31G*). The choice of the shape and geometric dimensions of the solute is more difficult. Following Wong et al. [14], a spherical shape was chosen for the amino acid analogues. Since most of the amino acids are small and relatively compact, this approximation is not unrealistic. On the other hand, the choice of the spherical radius is more crucial. However, for the purposes of conformational profiles, the relative energies were independent of this choice (manuscript in preparation). On the other hand, solvation energies (the energy difference between the neutral species in vacuo and the zwitterionic moiety in solution) are probably highly dependent on the choice of radius. The radii of the analogues were calculated by a quantum-mechanical approach based on the electron density envelope using the 6-31G* basis set [16] and the global energy minimum conformation as determined by the AM1 method in vacuo. The cavity radius was fixed at this calculated value during the optimizations. Although the radius does not vary when the C$^{\alpha}$-C[=O] torsion is changed, there is a small variation as a function of the C$^{\alpha}$-C$^{\beta}$ torsion. For the dielectric constant, the value for acetone, 20.7, was chosen. It is clear that the L-alanine receptor environment is only moderately polar, as evidenced by the fact that in

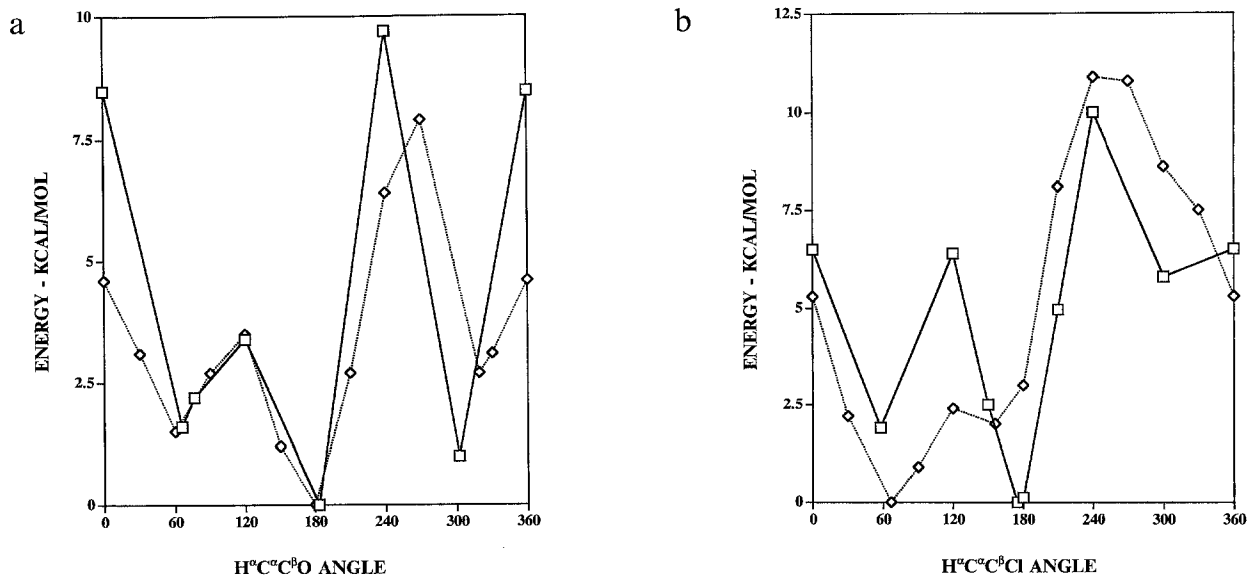


Fig. 2. Comparison of the AM1 (in vacuo) and SCRF-6-31G*//6-31G* ($\varepsilon = 20.7$) relative conformational energies of (a) the L-serine zwitterion as a function of the $H^{\alpha}C^{\alpha}C^{\beta}O$ torsion angle; and (b) the β-chloro-L-alanine zwitterion as a function of the $H^{\alpha}C^{\alpha}C^{\beta}Cl$ torsion angle. —□— = ab initio SCRF energy; ···◇··· = AM1 energy.

similar amino acid receptors [3,5] the medium surrounding the receptor's active site is less polar than an aqueous medium. As a result, the lowest energy conformers interacting with the receptor should be those conformers that are stable in a moderately polar environment. Although the value of the dielectric chosen here ($\varepsilon = 20.7$) is somewhat arbitrary, it is representative of a moderately polar medium. A study of the SCRF-631G* relative torsional energies of the high-affinity ligands in solvents with low and moderate dielectric constants ($\varepsilon = 2.0$ to 40.0) is now in progress (manuscript in preparation).

After determining the ab initio SCRF low-energy conformations in the appropriate receptor environment, the α-carbon, the carboxylate oxygens, the amino nitrogen and the carbonyl carbon of the analogues in their global minimum energy conformations were superimposed on the equivalent atoms of β-chloro-L-alanine, which is an analogue with one of the highest binding affinities. These superpositions were done to investigate the relative placement of the carboxylate oxygens of the analogues: Are they all in relatively the same region of space? In addition, the steric relationship of the substituent on the β-carbon (chlorine, hydroxy group, cyclopropane ring, etc.) to the zwitterionic moiety can easily be determined. Besides steric relationships, the question of the electrostatic components of the binding and the ultimate nature of activation can also be answered by this general approach. Since it is clear that the strength of binding is probably determined by the ionic interaction, the question remains as to whether the NH_3^+ and COO^- groups are in the correct position for maximum interaction. This question was partially answered by an investigation of the above superpositions in the region of the COO^- group since, as mentioned above, the NH_3^+ group can easily achieve correct alignment because of the low energy of the C^{α}-N torsion.

Results and Discussion

Experimental results for a series of ligands of high and moderate affinities are given in Table 1. The table shows that small substituents on the α-carbon produce the high-affinity ligands (analogues **1**, **2**, **3**, **4**, **5**, and **11**). On going from CH_2Cl (**2**) to CH_2CH_3 (**8**) to CH_2CN (**6**) and $CH{=}CH_2$ (**7**), the IC_{50} values increase. As was pointed out previously [4a], the IC_{50} values correlate well with molecular volume. For example, two substituents on the β-carbon produce ligands with very low affinity, e.g. **9**. Recently, L-valine (**10**) was tested and both its binding and neural response were similar to those of L-threonine (**9**). On the other hand, activation (in the form of neural response) is not equivalent to high binding affinity; see for example the results of analogues **3** and **4**. In addition, low binding affinity can occur alongside relatively high neural response (**7**).

Analysis of binding affinity

As mentioned above, the low-energy conformations for the analogues with the highest affinity (IC_{50} values lower than 4.0 μM) were determined by fully optimized ab initio SCRF 6-31G* analysis of the conformational energy spectrum. Figures 1a and b give plots of the relative conformational energy of glycine (**4**) and L-alanine (**1**) versus the H-C^{α}-C-O dihedral angle as determined by the AM1 method (in vacuo) and the ab initio SCRF method ($\varepsilon = 20.7$).

With the exception of the results for the global mini-

ma, the two methods predict somewhat different energy profiles for these two systems. Also, with the exception of L-serine (**3**), the ab initio global minimum for the C^{α}-C[=O] rotation for all of the analogues with high affinity is found at an N-C^{α}-C-O dihedral angle of 180° ± 5°. In every case, the ab initio and AM1 methods predict a similar value for the dihedral angle at this global minimum. In Figs. 2a and b, the ab initio 6-31G*//6-31G* plots of the torsional energy in L-serine and β-chloro-L-alanine (**2**) versus the H^{α}-C^{α}-C^{β}-X dihedral angles (X = O and Cl, respectively) are given.

The results of the AM1 calculations in vacuo are given for comparative purposes. Again, the ab initio and AM1 conformational energy profiles for the two systems are somewhat different. In particular, the global minimum for β-chloro-L-alanine (**2**), as determined by the two methods, is different. The ab initio calculation predicts a global minimum for **2** at an H^{α}-C^{α}-C^{β}-Cl dihedral angle of 174.9°, whereas the AM1 calculation predicts the global minimum to be at an H^{α}-C^{α}-C^{β}-Cl dihedral angle of 66.9°. It should be pointed out that, as the polarity of the medium drops from 20.7 (acetone) to 2.1 (hexadecane), the difference between the two lowest ab initio SCRF minima (at approximately 60° and 180°) in **2** drops from approximately +2 kcal/mol to −0.4 kcal/mol. Interestingly, the ab initio SCRF results at ε = 2.1 are now more in line with the AM1 in vacuo results. This result clearly indicates that some of the difference between the two methods is due to solvent effects and not due simply to an inadequacy in the AM1 method. A further discussion of solvent effects on conformational energy profiles will appear in a future publication. Finally, the results from the ab initio calculations indicate a very narrow range for the carbonyl carbon–amino nitrogen distance (2.46–2.54 Å) in each of the ligands with high affinity. This restricted distance is, in fact, found in almost all of the ligands analyzed, including some of the ligands with moderate affinity (**8** and **14**).

The global minima as determined by the ab initio 6-31G*//6-31G* calculations served as the input for the superposition diagrams. The template was chosen as the conformation corresponding to the global energy minimum of β-chloro-L-alanine (**2**), which ranks as one of the ligands with highest affinity. L-alanine and L-serine (Fig. 3a) as well as the cyclopropane derivative (**11**) (Fig. 3b) were superimposed onto β-chloro-L-alanine in the manner outlined above.

As can be seen from the diagram, the three systems do occupy almost the same region of space. To indicate this common space, a Connolly surface [21] of β-chloro-L-alanine is shown in Fig. 4. Here, the outer surface should yield a good representation of the receptor's complementary surface, with positive residues interacting with the carboxylate moiety and negative residues interacting with the charged amino group. Thus, the Connolly surface can be used to represent the complementary surface of the receptor [21]. Analogues whose side chains intrude on this surface, especially in the region around the $-CH_2Cl$ group (the 'hydrophobic pocket'), would most likely not be ligands with a high affinity for the receptor site. Thus, as the size of the substituent increases ($-CH_3$, $-CH{=}CH_2$, CN), the binding affinity decreases. The relatively high affinity associated with compound **8** may be due to the flexibility of the terminal methyl group and its ability, energetically, to occupy a relatively optimal position in space for binding. The slight differences in the IC_{50} values of the ligands with the highest affinity are most likely due to small differences in the energetics of their interactions with the positive and negative receptor residues.

Analysis of neural response (activation)

The substituents on the β-carbon as well as the volume of the region around this carbon are obviously crucial for activation and it is likely that dispersion forces are operative in this region, with stabilization and eventual activation possibly occurring through the macroscopic hydrophobic effect. One approach to testing this hypothesis would be to correlate the free energy term associated with dispersion interactions and cavity formation with the analogue's neural response.

As mentioned in the methodology section, the AM1-SM2 model computes a free energy term (G°_{CDS}) that contains terms associated with the formation of the solute cavity, a dispersion interaction term, and a solvent reorganization term. Of considerable interest here is the difference between G°_{CDS} in water and in the receptor environment, i.e., the free energy change associated with the transfer process analogue (water) → analogue (less polar receptor environment):

$$\Delta G^{\circ}_{CDS} = G^{\circ}_{CDS}(\text{receptor environment}) - G^{\circ}_{CDS}(\text{water}) \quad (1)$$

This particular free energy term involves the energy associated with the transfer of the amino acid from water to the neighborhood of the receptor, with its relatively non-

TABLE 2
G°_{CDS}(WATER) AND ΔG°_{CDS}(REL)[a] FOR SELECTED ANALOGUES

Analogue	G°_{CDS} (water) (kcal/mol)	ΔG°_{CDS} (rel) (kcal/mol)
1	−5.2	−1.1
2	−5.3	−1.0
3	−8.3	2.0
4	−6.3	0.0
5	−4.3	−2.0
6	−7.7	1.4
7	−5.0	−1.3
8	−4.9	−1.4
11	−4.9	−1.4
14	−4.4	−1.9

[a] Free energy relative to glycine.

polar environment around the residue's β-carbon. In the first column of Table 2, the value of G°_{CDS}(water) for a series of analogues as computed by the AM1-SM2 method [12] is given. The conformations used were similar to the AM1-determined global energy minimum structures in vacuo. However, for most of these species, using other conformations does not alter the results. Since the area around the β-carbon is assumed to be primarily responsible for activation and neural response, the contribution from that region to the total value of G°_{CDS}(water) is of considerable interest. Subtracting the value of G°_{CDS}(water) for glycine from that of the particular analogue should give a rough estimate of the contribution of the area around the β-carbon in the analogue to G°_{CDS}(water), or the relative contribution to G°_{CDS}(water). Now, assuming that the first term on the right-hand side of Eq. 1 represents a less polar environment than water and its relative value can be ignored, then, to a first approximation, ΔG°_{CDS}(rel) can be determined from the negative of the relative contribution to G°_{CDS}(water). Thus, in the second column of Table 2, the values of ΔG°_{CDS}(rel) are given. For the most active analogues the value is negative, indicating a favorable ΔG°_{CDS}(rel). With the exception of analogue **8**, all the compounds which are formed from side-chain modifications of L-alanine and have a neural response greater than 70% also have a negative value for ΔG°_{CDS}(rel). Furthermore, the cyclopropane derivative, which is an excellent activator, displays a negative and favorable value for this term. In addition, analogues that are somewhat sterically inhibited from binding (**7** and **8**) have negative values and are relatively active, presumably because of a favorable cavity dispersion term. On the other hand, L-serine (**3**), a high-affinity binder but only a relatively fair activator, has an unfavorable, i.e. positive, value for this term. For analogues **1**–**7** and **11**, a least-squares linear regression plot yields a correlation coefficient r of 0.838 for the two variables neural response and ΔG°_{CDS}(rel). However, since only a small set of compounds is utilized in this analysis, this result may not be significant. Obviously, as larger nonpolar groups are substituted at the β-carbon, steric interference would eliminate a high neural response, despite a favorable free energy term.

Finally, it is important to point out the effects of achiral side-chain modifications of L-alanine. Analogues such as D-serine (**13**) and D-alanine (**12**), which should have ΔG°_{CDS} values identical to those of their L-counterparts, show moderate binding affinity but, in the case of **12**, are poor activators. Likewise, 2-amino-2-methylpropanoic acid (**14**) is not a high-affinity binder like glycine or L-alanine. In this case, it is clear that achiral modification affects the binding affinity. An unfavorable disposition of bulk might now be affecting the directionality of the zwitterionic interactions with the receptor. In addition, despite presumed favorable free energy terms, the neural response is low for these three analogues, indicating that substitution of another methyl group at the α-carbon also reduces the activity of the analogue. Thus, the above analysis is limited to rationalizing the neural response of analogues with side-chain modifications. No doubt part of this limitation is due to the continuum solvent models themselves, which do not take into account the naturally chiral environment of the receptor.

Conclusions

The interaction between the analogues and the L-alanine receptor can be described as a combination of two terms, i.e., $E_{binding} = E_{ionic} + E_{residue}$, where the energy due to ionic binding, the largest binding term, is most likely common to all amino acid receptors and is due to the ionic interaction of the zwitterionic moiety with complementary sites on the receptor. A zwitterionic analogue would be a good ligand, given the correct steric dimensions as described by the surface map of β-chloro-L-alanine. As the number and size of substituents on the β-carbon increase, the binding affinity drops rather dramatically. The second term, the energy term due to interactions involving the residue on the analogue, is most likely a much smaller energy term but probably plays a dominant role in activation at the L-alanine receptor. Analogues that are characterized by free energy hydrophobic-type interactions similar in magnitude and location to the best binders such as β-chloro-L-alanine and L-alanine should, given the presence of a zwitterion and the correct steric requirements as defined above, be not only high-affinity binders, but excellent activators as well. One such molecule, which we tested experimentally as a result of this analysis, is β-fluoro-L-alanine (**5**). This analogue superimposes well with those analogues with the highest affinity and its relative cavity dispersion free energy term is negative (see Table 2) and also similar in value to the comparable terms for the high-affinity binders. As predicted, its IC_{50} and neural response are comparable to those of L-alanine. Although one might expect the fluorine analogue to be potent, in other systems (such as the amiloride analogues [9]) with different modes of activation, the fluorine analogue is less active than the chlorine analogue. Finally, other potentially high-affinity analogues might be those which incorporate an oxygen into the cyclopropane ring, such as the epoxides.

Although the molecular modeling analysis of the L-alanine receptor in the channel catfish presented here suggests a rationale for both binding and activation, the question of the exact nature of the activation step remains. The free energy term G°_{CDS}, calculated by the AM1-SM2 method, is empirically determined and, although reasonable for qualitative comparison, it is less quantitatively reliable [22]. In addition, use of recent extensions of the AM1-SMx method by Cramer and Truhlar [13] to the calculation of G°_{CDS} in hexadecane

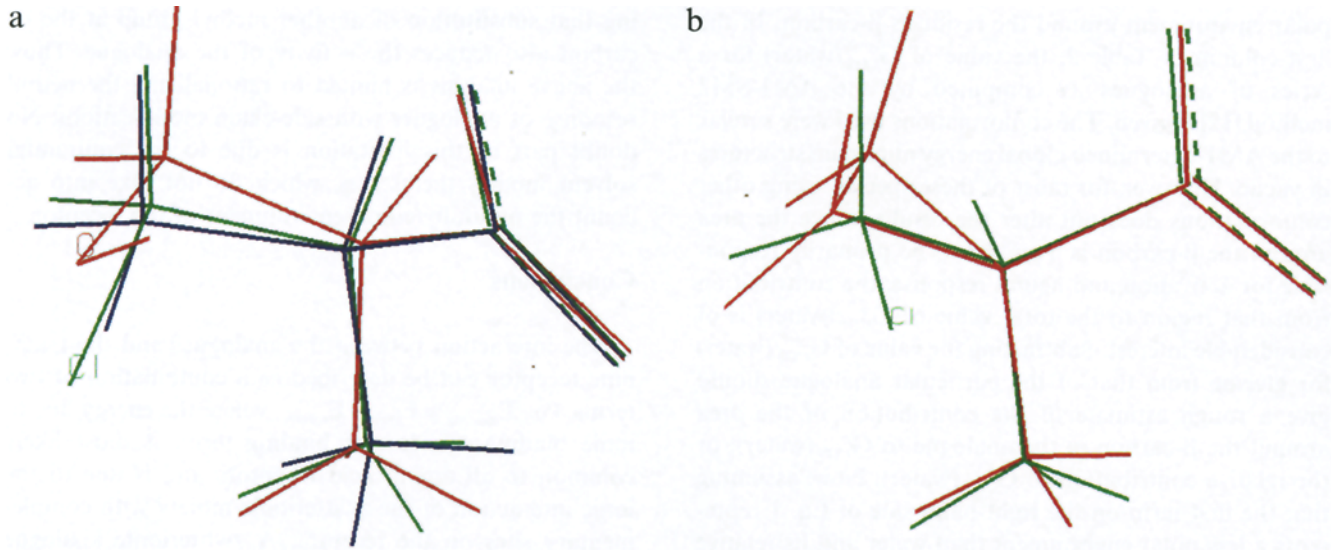


Fig. 3. (a) Superposition of the 6-31G*//6-31G* energy-minimized structures of the zwitterions of L-alanine (blue) and L-serine (red) onto the zwitterion of β-chloro-L-alanine (green). The oxygen of the -OH group in L-serine and the chlorine in β-chloro-L-alanine are labelled. (b) Superposition of the 6-31G*//6-31G* energy-minimized structure of the zwitterion of 1-aminocyclopropane carboxylic acid (red) onto the zwitterion of β-chloro-L-alanine. The chlorine in β-chloro-L-alanine is labelled.

(AM1-SM4 model) would allow a more accurate determination of ΔG°_{CDS} (rel). A careful analysis of a model of the interaction of the substituent group at the β-carbon with a reasonable model of the relevant interacting residue on the receptor (phenylalanine, tyrosine, etc.) should, along with the results of the above hydrophobic model, produce interaction energies that correlate more closely to the experimental data. In any case, the hydrophobic model presented here is a good starting point, not only for understanding the mechanism at this specific amino acid receptor, but also for understanding the binding at other, more complex amino acid receptors.

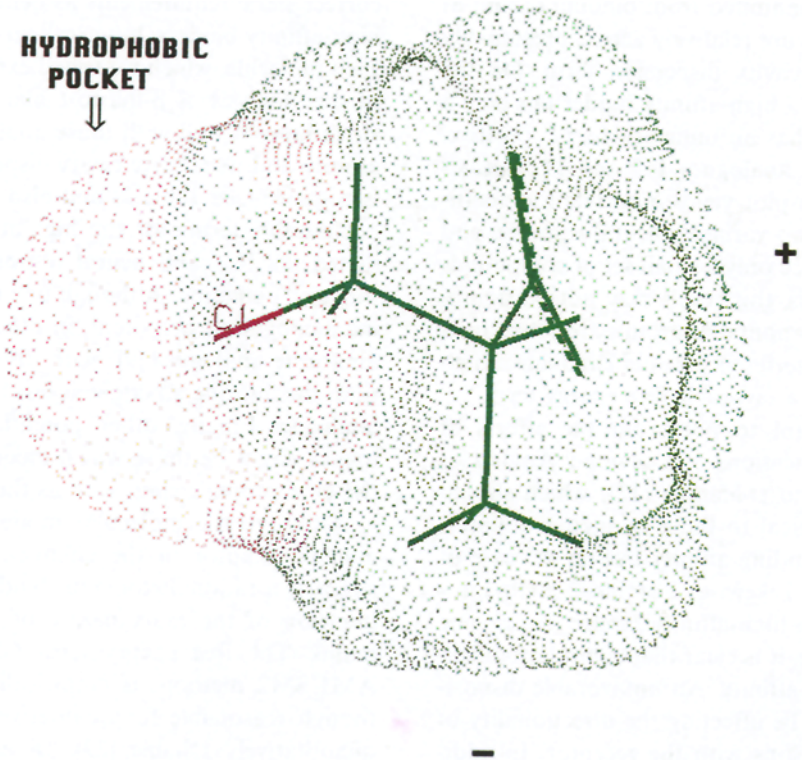


Fig. 4. Connolly surface of β-chloro-L-alanine, showing putative positive (+) and negative (−) sites on the receptor as well as the hydrophobic pocket.

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