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Conformational analysis of kainate in aqueous solution in relation to its binding to AMPA and kainic acid receptors

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Summary

Conformational analyses for kainate in aqueous solution have been performed by using the MM3*, AMBER* and MMFF94 force fields in conjunction with the Generalized Born Solvent Accessible Surface (GB/SA) hydration model. A comparison of calculated results with experimentally determined conformational data indicates that MM3*-GB/SA strongly overestimates the stability of a hydrogen bonded ion-pair in aqueous solution in comparison with the separated and solvated ions. This results in an incorrect prediction by MM3* of the most stable conformer of kainate in aqueous solution, whereas AMBER* and MMFF94 correctly predict the lowest energy conformer. Calculated conformational energy penalties for binding of kainate to the AMPA iGluR2 receptor indicate that the lower affinity of kainate for AMPA receptors compared to its affinity for kainic acid (KA) receptors is not due to a higher energy bioactive conformation of kainate at AMPA receptors. This conclusion is strongly supported by an analysis of a recently reported nonselective AMPA/KA ligand and a comparison of the conformational and structural properties of this ligand with iGluR2-bound kainate. This comparison strongly suggests that kainate binds to AMPA and KA receptors in closely the same conformation.

Introduction

Kainic acid (Figure 1) has given name to a class of ionotropic glutamate receptors, the kainic acid (KA) receptors. Ionotropic glutamate receptors (iGluRs) mediate excitatory synaptic transmission in the central nervous system through ligand induced opening of transmembrane ion channels. These receptors are important in many processes of the central nervous system, e.g. in memory and learning and are implicated in diseases such as Alzheimer's, Parkinson's and Huntington's as well as schizophrenia, epilepsy and CVA [1].

Kainic acid is selective for KA receptors and is the standard ligand for pharmacological studies of these receptors. However, kainic acid also binds and activates another class of ionotropic glutamate receptors, the AMPA receptors named after the selective agonist (S)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid ((S)-AMPA (Figure 1)). Although kainic acid displays a lower affinity for AMPA receptors than for KA receptors by a factor of 600 [2], it is often used as ligand in studies of AMPA receptors due to its non-desensitizing properties at these receptors [1].

The molecular determinants for the selectivity of kainic acid for KA receptors with respect to AMPA receptors is presently not understood. This is unfortunate as lack of selective ligands for KA receptors have hampered the pharmacological characterization of these receptors [1]. Possible reasons for the selectivity of kainic acid for KA receptors include (i) stronger interactions of kainic acid with amino acid residues in the ligand binding site of KA receptors, (ii) steric repulsions in the binding site of AMPA receptors and/or steric attractions in the KA binding site and (ii) dif-

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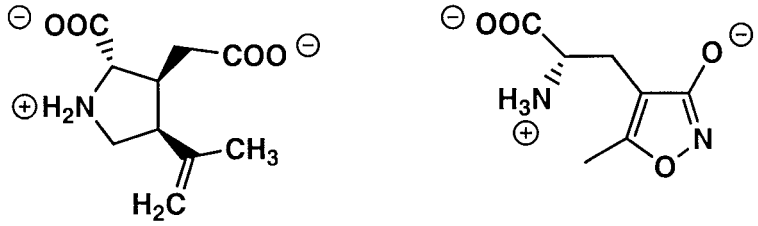


kainate **(S)-AMPA**

Figure 1. Structures of kainic acid and (S)- AMPA in their protonation states at pH = 7.

ferent conformations of kainic acid in the two binding sites with a higher energy conformation in the AMPA site.

The AMPA receptors include four receptor subunit building blocks, iGluR1-4 and the receptors have a heterotetra- or pentameric structure [1]. Kainic acid displays very similar affinities for the different AMPA receptor subunits [3]. Recently, the crystal structure of the AMPA receptor iGluR2 ligand-binding domain in complex with kainate was reported [4]. For the first time, this makes it possible to directly study the interactions between a ligand and an iGluR receptor. Structural information on kainate in complex with KA receptors is at present not available. Whereas such information is required to study possible differences in the interactions of kainate with the binding sites of the two classes of receptors (AMPA and KA), it is possible to investigate if the conformational energy penalty for binding of kainic acid to the iGluR2 receptor may be a reason for its low affinity for the AMPA receptors. The four subunits of the AMPA receptor are highly homologous (70–80% sequence identity) and the key residues of the binding site are identical in the subunits [1]. This makes it highly probable that the bioactive conformation of kainate is the same at all AMPA subunits.

In the present work we have performed conformational analysis of kainate in aqueous solution using the MM3*, AMBER* and MMFF94 force fields in conjunction with the Generalized Born/Solvent Accessible Surface (GB/SA) hydration model. The energy of the kainate conformation as displayed by the experimentally determined iGluR2-kainate complex has been compared with the lowest energy conformer found by conformational analyses in aqueous solution. As experimental conformational data for kainate in aqueous solution obtained by NMR spectroscopy have been reported [5], conformational analyses using different force fields also provide an opportunity to evaluate the performance of these force fields used in conjunction with the GB/SA hydration model.

Previously reported conformational analyses of kainic acid have all been confined to its zwitterionic form with a protonated ω-carboxylate group either in vacuo[2]or employing explicit water molecules (refered to by Todeschi et al. in ref. 5, but no conformational energy data from the these calculations have so far been reported). It should be noted that these calculations do not refer to the protonation state of kainic acid at physiological pH. The pK_as of kainic acid are 2.1 (α-CO_2H), 4.3 (ω-CO_2H) and 10.1 (NH_3^+) [6]. Thus, at pH ≈ 7 both carboxylic acids are deprotonated and NH_2 protonated and kainic acid is in its triply ionized form (Figure 1). This is also the protonation state of kainate in complex with iGluR2 [4] and this protonation state has been used throughout the present study.

Computational methods

Force fields and hydration model

All calculations were performed by using the MM3*, AMBER* and MMFF94 force fields as implemented in MacroModel version 6.5 [7]. For the aqueous phase calculations, the Generalized Born Solvent Accessible Surface (GB/SA) hydration model [8] in the MacroModel program was employed.

During the course of the calculations, a problem in the MM3* force field in connection with calculations on carboxylate ions was encountered. In the Macromodel program, a carboxylate ion is constructed from the carboxylic acid by deleting the hydrogen of the OH group and adding a negative charge to the oxygen. Thus, the two carboxylate oxygens get different atom types (OM and O2). The program automatically

equilibrates the charges of the two carboxylate oxygens and makes the stretching parameters for the two C-O bonds equal, but it does not equilibrate the rest of the force field, e.g., bending and torsion. Thus, calculations on the two degenerate rotamers of the carboxylate group (generated by a 180 deg rotation about the C-CO_2^- bond) may give different MM3* energies. To solve this problem we have added parameters to the MM3* force field to ensure equality of the two carboxylate oxygens of the carboxylate group. The added force field parameters (taken from MM3(94) [9] or estimated) are given in the Appendix. It should be noted that this problem does not occur in the AMBER* and MMFF94 force fields.

Semi-empirical calculations

A few calculations were performed by using the AM1/SM2 (SM2.1) and AM1/SM5.4 (SM5.4A) methods as implemented in AMSOL [10].

Constrained relaxation of receptor-bound kainate

Crystallographically determined ligand-protein structures contain uncertainties which may result in significant errors when the energies of an energy minimized ligand structure and the corresponding structure in an experimentally determined ligand-protein complex are compared. These errors are especially pronounced for the uncertainties in bond lengths for which the large force constants for bond stretching/compression may severely distort the energy calculations. In order to eliminate these incompatibilities, a partial relaxation of the X-ray structure within each force field is necessary [11].

The coordinates for kainate in its complex with iGluR2 was extracted from the Protein Data Bank (PDB-ID 1GR2) [12]. After the present work was completed the coordinates of two more kainate-iGluR2 complexes have become available (PDB-ID 1FTK and 1FW0). The structures of kainate in the three experimental complexes are virtually identical.

Hydrogen atoms were added to kainate and for each force field flat-bottomed Cartesian constraints as implemented in the MacroModel program were used to partially relax the experimental structure. All non-hydrogen atoms were tethered to their crystallographic positions during the optimization by harmonic flat-bottomed constraints. The flat-bottomed radius, i.e. the distance the heavy atoms are allowed to move from the X-ray structure without an energy penalty was set to 0.3 Å. At larger distances a harmonic energy penalty function with a force constant of 120 kcal/mol $Å^2$ (500 kJ/mol $Å^2$) was applied. The flat-bottomed relaxation was performed with and without inclusion of GB/SA water. As the in vacuo calculations resulted in severe distortions of the molecular geometry, these were discarded and only the results from the partial relaxations in aqueous phase were used in the present study.

Conformational analysis

The conformational space of kainate was searched for low energy conformations in aqueous solution (GB/SA) by using the Monte Carlo multiple minimum (MCMM) method [13], implemented in the MacroModel program. The conformational searches were continued until all low energy minima had been found multiple times. All non-hydrogen atoms were superimposed in the test for duplicate structures. The energy minimizations were carried out using the truncated Newton conjugate gradient (TNCG) algorithm.

Prior to the conformational search on kainate, a conformational search was for each force field performed for the (2*R*,3*S*, 4*R*)-2,3,4-trimethyl-pyrrolidinium ion (i.e., the five-membered ring system of kainate with methyl groups at the 2, 3 and 4 positions). In this way all possible ring conformers of the pyrrolidinium ion was generated. In the following step, the methyl substituents in each of the calculated conformers of the (2*R*,3*S*, 4*R*)-2,3,4-trimethyl-pyrrolidinium ion were replaced by the appropriate substituents to convert it to the kainate structure. Each of these kainate conformations, two generated by MM3* and MMFF94 and three generated by AMBER* was used as starting structures for the final conformational search of kainate with respect to all rotatable bonds using an energy cutoff of 4.8 kcal/mol (20 kJ/mol) above the global energy minimum.

Calculation of the conformational energy penalty for ligand binding

The total free energy of binding may be written as:

$$\begin{aligned}\Delta G \;=\; & \Delta G_{\text{inter}} + \Delta G_{\text{conf}}(\text{ligand}) \\ & + \Delta G_{\text{conf}}(\text{protein}) + \Delta G_{\text{solv}},\end{aligned}$$

where ΔG_{inter} is the in vacuo energy required to separate the ligand and the protein as rigid molecules, ΔG_{conf}(ligand) the in vacuo conformational energy difference between bound ligand and the conformational ensemble in aqueous phase, ΔG_{conf}(protein)

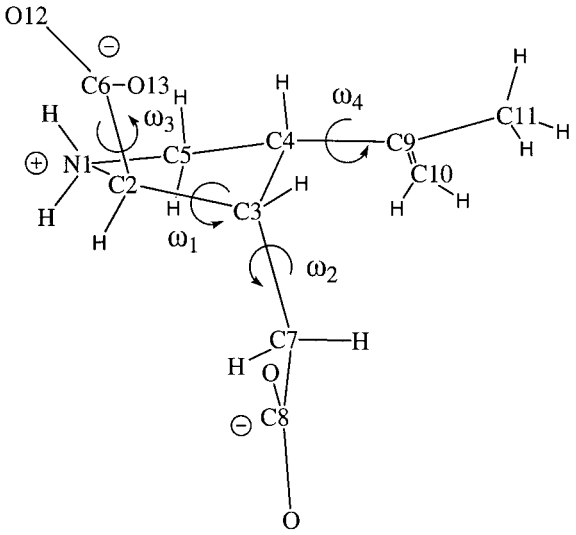


Figure 2. Atom numbering and definition of the dihedral angles $\omega_1 - \omega_4$. ω_1 = C6-C2-C3-C7; ω_2 = C2-C3-C7-C8; ω_3 = O12-C6-C2-N1; ω_4 = C10-C9-C4-C5.

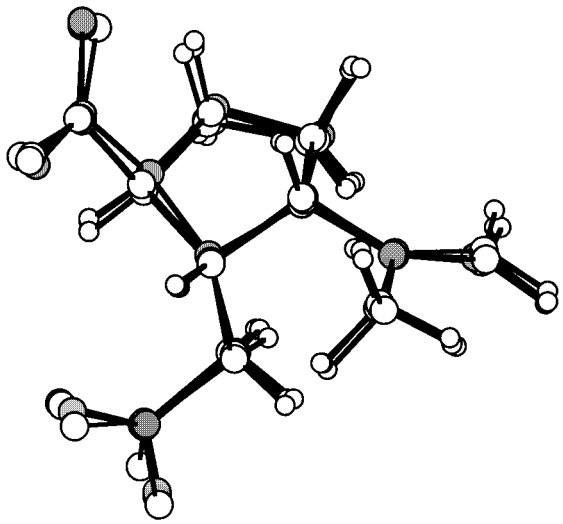


Figure 3. Least-squares superimposition of the unmodified X-ray structure of kainate in the iGluR2-kainate complex (filled atoms) and the partially relaxed structures obtained by MM3*, AMBER* and MMFF94 (unfilled atoms) using flat-bottomed Cartesian constraints.

the corresponding conformational energy difference for the protein and ΔG_{solv} the overall change in solvation energy for the process, i.e. ΔG_{solv}(complex) - ΔG_{solv}(ligand) - ΔG_{solv}(protein) [11]. Neglecting conformational entropies, the conformational energy penalty for ligand binding (ΔG_{conf}(ligand)) may be estimated by subtracting the internal (steric) energy of the preferred conformation in aqueous solution, (i.e., the energy of the global energy minimum in aqueous solution excluding the hydration energy) from the calculated energy of the bound ligand after flat-bottomed constrained structure optimization (excluding the hydration energy). This procedure have previously been employed in a study of conformational energy penalties for a large number of protein-bound ligands [11].

Characterization of ring and substituent conformations

The different conformers of the pyrrolidinum ringsystem of kainate may be characterized as having either an envelope (E) or a twist (T) form. By viewing the ring in the orientation shown in Figure 2, the ring conformation may be defined by using superscripts and subscripts to designate ring atoms which are up or down, respectively, with respect to the overall plane of the ring [5, 14]. In substituted five-membered rings as in kainate, ideal envelope and twist structures are unlikely due to substituent interactions. In this work, the ring conformation is characterized by the nearest conformational type. A ring structure which have one dihedral angle angle less than 3 deg (ideally zero degrees) is characterized as an envelope (E) conformation. For instance, the ring conformation of kainate in complex with the iGluR2 (Figure 2) is characterized as $^4T_3$. The dihedral angles defining the conformation of the glutamate part of kainate and the orientation of the isopropenyl substituent at C4 are denoted by ω_1-ω_3 and ω_4, respectively (Figure 2).

Results

Constrained optimizations of kainate

As shown in Table 1, the ring conformation of kainate as observed in the kainate-iGluR2 complex ($^4T_3$) is preserved in the flat-bottomed optimizations by all three force fields and only small changes of dihedral angles of the substituents are observed. The rms values in Table 1 obtained by superimposing all heavy atoms reflect the minor structural differences between the flat-bottomed optimized structures and the experimentally determined kainate structure. A superimposition of the experimental structure and the flat-bottomed optimized structures is shown in Figure 3.

Table 1. Ring structures and dihedral angles $\omega_1 - \omega_4$ (in degrees) for kainate in the iGluR2-kainate complex and for the X-ray structure partially relaxed by MM3*, AMBER* and MMFF94 using flat-bottomed Cartesian constraints

	Ring structure	ω_1	ω_2	ω_3	ω_4	R.m.s. (Å)
X-ray structure	$^4T_3$	151.9	−79.1	11.8	−11.8	
MM3*	$^4T_3$	154.5	−71.1	15.3	−3.7	0.148
AMBER*	$^4T_3$	156.1	−64.2	2.4	3.0	0.203
MMFF94	$^4T_3$	151.9	−70.1	12.9	−2.2	0.118

Table 2. Calculated conformational energies (in kcal/mol) for kainate in aqueous solution

Conformer No.	Relative MMFF94* energy[a]	Relative energy of hydration	Relative internal energy
1	0.0 (96)	0.0	0.0
2	2.4 (2)	−41.3	43.7
3	2.8 (1)	−47.8	50.7
4	2.9 (1)	−41.7	44.6
5	3.0 (1)	−50.2	53.2
Conformer No.	**Relative AMBER* energy[a]**	**Relative energy of hydration**	**Relative internal energy**
1	0 (56)	0	0
2	0.7 (17)	25.6	−24.9
3	0.8 (14)	−0.2	1.1
4	1.0 (10)	25.9	−24.9
5	1.8 (2)	1.5	0.3
6	2.2 (1)	23.5	−21.4
7	2.9 (<0.5)	23.9	−21.1
Conformer No.	**Relative MMFF94* energy[a]**	**Relative energy of hydration**	**Relative internal energy**
1	0 (54)	0	0
2	0.5 (24)	0.7	−0.2
3	0.7 (16)	30.4	−29.6
4	1.9 (2)	−0.3	2.2
5	2.1 (2)	−0.6	2.7
6	2.4 (1)	1.1	1.3
7	2.6 (1)	30.7	−28.1
8	2.6 (1)	−0.2	2.8

[a]The corresponding Boltzmann distribution (in %) is given within parentheses.

Conformational analysis of kainate in aqueous solution

As expected, conformational searches in vacuo employing the MM3*, AMBER* and MMFF94 force field in each case gives a global energy minimum displaying a strong intramolecular ion-pair hydrogen bond between the ammonium group and the carboxymethyl substituent at C3. A comparison of the internal energies between the receptor-bound structure (flat-bottom optimized) and the global energy minimum *in vacuo* gives for all force fields an energy difference of more than 25 kcal/mol. Such large energy penalties for binding are clearly not compatible with the affinity of kainate for the iGluR2 receptor. As previously discussed [11], this underlines the necessity of performing the conformational analysis for aqueous phase if conformational penalties for receptor binding are to be calculated.

The results of the conformational searches for aqueous solution are given in Table 2. The Table includes the calculated relative conformational energies and the corresponding Boltzmann distributions for all conformers with a calculated energy of less than 3 kcal/mol above the global energy minimum. In addition, the relative calculated hydration energies of the conformers and the relative internal energies (hydration energies excluded) are given. The type of pyrrolidinium ring structure and the calculated dihedral angles $\omega_1 - \omega_4$ are given in Table 3. The calculated lowest energy conformer for each force field is shown in Figure 4. They all display the same type of ring conformation ($^4T_3$) as is also observed in receptor-bound kainate. As expected, they all display a hydrogen bond between the C2 carboxylate group and the ammonium ion. The main difference between the calculated lowest energy conformations in Figure 4 is the orientation of the carboxymethyl group at C3.

*MM3**

MM3* gives five conformers within an energy of less than 3.0 kcal/mol above the global energy minimum (Table 2). The global energy minimum (Figure 4)

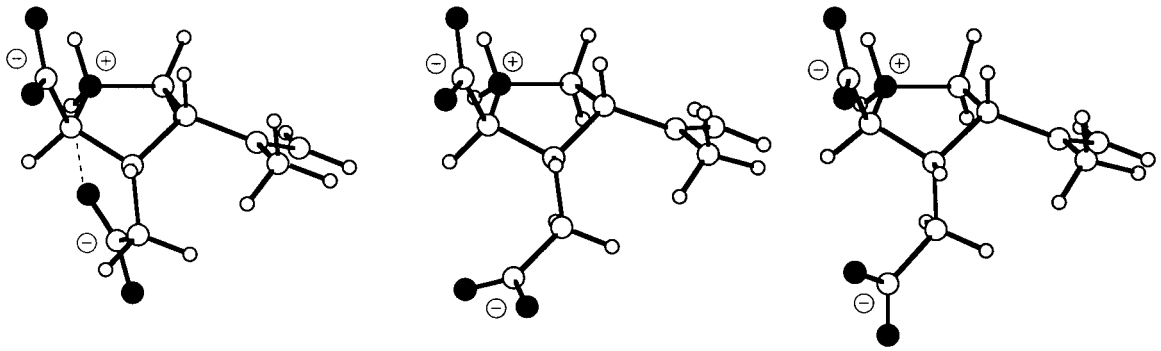


Figure 4. Calculated lowest energy conformations for kainate in aqueous solution.

Table 3. Geometrical parameters for the calculated low energy conformers of kainate in aqueous solution. Dihedral angles $\omega_1 - \omega_4$ in deg

Conf.	MM3*					AMBER*					MMFF94				
No.	Ring structure	ω_1	ω_2	ω_3	ω_4	Ring structure	ω_1	ω_2	ω_3	ω_4	Ring structure	ω_1	ω_2	ω_3	ω_4
1	$^4T_3$	152.0	38.4	10.4	−5.0	$^4T_3$	153.5	−68.9	−1.1	3.2	$^4T_3$	151.9	−70.1	13.0	−2.2
2	$^4T_3$	154.5	−71.1	15.4	−3.7	E_3	156.1	52.8	10.5	0.6	$^4T_3$	148.2	−71.8	5.5	−2.5
3	^4E	143.7	−70.3	64.6	−4.8	$^4T_3$	152.1	−65.3	−1.1	148.6	$^2T_3$	149.0	56.2	17.5	−15.1
4	E	148.0	−69.2	6.3	−4.6	E_3	154.7	49.6	10.4	148.8	$^4T_3$	151.4	−68.2	13.3	157.1
5	$^4T_3$	139.6	−69.8	78.1	−5.5	$^4T_3$	156.3	−68.6	1.3	−154.8	$^4T_3$	150.8	−75.3	7.3	−148.9
6						E_3	168.3	−11.2	13.6	11.3	$^2T_3$	148.3	52.1	16.3	153.5
7						E_3	169.1	−12.1	13.5	−141.7	$^4T_3$	148.0	−68.7	5.9	156.5
8											E_4	85.9	−172.1	4.5	156.6

is calculated to be the only significantly populated conformer. It is characterized by a strong ion-pair hydrogen bond between the ammonium group and the carboxymethyl group at C3 (indicated by a dashed line in Figure 4). This conformation is similar to the one calculated to be the global energy minimum in vacuo for all three force fields used in this study. Conformers 2–5 all display a dihedral angle ω_2 similar to the one observed in the protein-bound structure (Tables 1 and 2, Figure 3) in which no hydrogen bond is formed between the ammonium and the carboxymethyl groups. Thus, with MM3* in combination with the GB/SA hydration model, the solvated hydrogen bonded ion-pair (Figure 4) is favored over conformers in which the ions are separated and solvated. The calculated GB/SA hydration energy for the global energy minimum is 41–50 kcal/mol less negative than for conformers 2–5 but this is more than compensated for by the stronger internal energy in the global energy minimum by 44–53 kcal/mol due to the ion-pair hydrogen bond (Table 2).

*AMBER**

The AMBER* force field in combination with GB/SA gives seven low energy conformations within the energy cut off of 3.0 kcal/mol (Table 2). The four lowest energy conformers have energies within 1.0 kcal/mol making up 97% of the conformational ensemble. Conformers 1, 3 and 5 display the $^4T_3$ type of ring conformation, whereas conformers 2, 4, 6 and 7 all display an E_3 conformation (Table 2).

The main structural difference between conformers 1 and 3 is the orientation of the C4 isopropenyl substituent (ω_4). The lowest energy conformer displays the same orientation of the C4 substituent as is observed in the bioactive conformation of kainate. The same structural difference is found for conformers 2 and 4. The most important conformational difference

between conformers 1/3 and 2/4 is the orientation of the carboxymethyl group (dihedral angle ω_2). In 1 and 3, this dihedral angle corresponds to the one found in the experimental protein-bound structure, whereas conformers 2 and 4 display an internal hydrogen bond between the ammonium group and the carboxylate ion in the C4 substituent as in the global energy minimum calculated by MM3* described above.

Conformers 2 and 4 display lower relative internal energies by 25 kcal/mol compared to 1 and 3 (Table 2) due to the internal ion-pair hydrogen bond in 2 and 4. However, in contrast to what was found by MM3*, the stronger hydration of the separated ammonium and carboxylate ions in 1 and 3 compared to the hydration of the hydrogen bonded ion-pair in 2 and 4 results in an lower total energy of 1 compared to 2 and of 3 compared to 4.

MMFF94

MMFF94 gives eight conformers within 3 kcal/mol above the global energy minimum (Table 2). The three lowest energy conformers make up 94% of the conformational ensemble. The lowest energy conformer (Figure 4, Tables 1 and 3) is virtually identical to the flat-bottomed optimized bioactive structure and conformer 2 is structurally very similar to 1 (Table 3). In both these conformers, the ammonium group and the carboxylate of the carboxymethyl group at C3 are separated and do not engage in hydrogen bonding and they are thus similar to the lowest energy structure found by the AMBER* force field (Figure 4). Conformer 3 with a conformational energy of 0.7 kcal/mol adopts a $^2T_3$ ring conformation and the C3 carboxymethyl is hydrogen bonded to the ammonium ion.

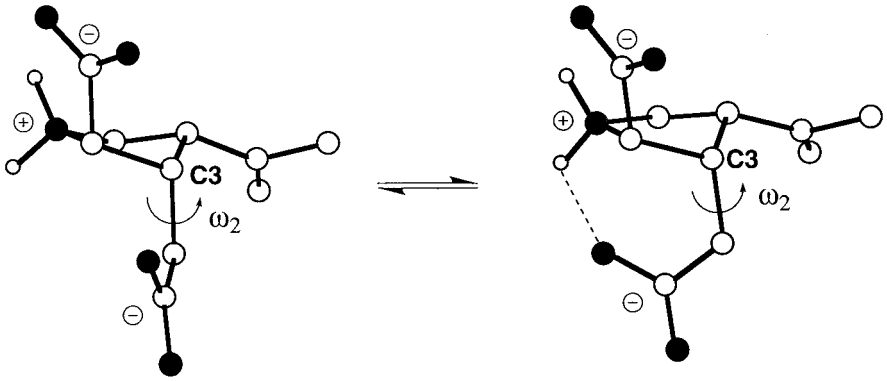
The structural similarity of conformers 1 and 2 is reflected in the similar relative energies listed in Table 2. Conformer 3 displays a larger internal energy stabilization by 29-30 kcal/mol due to the hydrogen bond between the carboxymethyl carboxylate and the ammonium ion. However, as with AMBER* but in contrast to MM3* this hydrogen bond results in less stabilization by the solvent compared to the separated and solvated ions.

Discussion

Comparison with experimental data for aqueous solution

The most important difference in the results obtained by the three different force fields in combination with the GB/SA hydration model is how they predict the relative stabilities of conformers of type I and II illustrated in Figure 5. In conformers of type I the carboxylate of the carboxymethyl group and the ammonium ion do not form a hydrogen bond, whereas in type II a strong ion-pair hydrogen bond is formed. As shown in Table 2, this strongly stabilizes the molecule but also results in less stabilization by the solvent. The resulting relative conformational energies in aqueous solution thus depends on the balance between these two energy contributions. With MM3*, the strongly dominating global energy minimum is predicted to be of type II. Low energy conformers of type I all have a conformational energy that is higher by at least 2.4 kcal/mol (Table 2). In contrast, AMBER* and MMFF94 predict a global energy minimum of type I, whereas for both force fields the lowest energy conformer of type II has a higher conformational energy by at least 0.7 kcal/mol.

Todeschi et al. [5]have studied the conformations of kainate in aqueous solution at neutral pH by ^{13}C and ^1H NMR. On the basis of H-H and H-^{13}C coupling constants and NOE experiments, the authors conclude that predominating conformation with respect to the dihedral angle ω_2 is gauche (−) ($\omega_2 \approx -60$ deg), i.e of type I in Figure 5, whereas a minor part of the conformational ensemble may consist of conformers displaying a dihedral angle ω_2 of ca. 180 deg. Most importantly, the experimental conformational analysis does not show any significant contribution of conformers containing a hydrogen bond between the carboxylate of C3 carboxymethyl group and the ammonium group as in type II. Such conformers (gauche (+)) are characterized by ω_2 of about 30–60 deg. As described above, 96% of the MM3* conformational ensemble consists of such hydrogen bonded conformers, whereas the AMBER* and MMFF94 ensembles only contain 28% and 17% , respectively, of the hydrogen bonded conformer (Table 2). These results indicate that MM3* in combination with GB/SA in this case strongly overestimates the stability of a hydrogen bonded ion-pair in aqueous solution in comparison with the separated and solvated ions. On the basis of the experimental data described above it is



I **II**

Figure 5. The equilibrium between a conformer without hydrogen bonding interactions between the C3 carboxymethyl group and the ammonium ion (**I**) and a corresponding conformer displaying an intramolecular ion-pair hydrogen bond (**II**). Hydrogen atoms (except on nitrogen) have been removed for clarity.

likely that also AMBER* and MMFF94 display the same overestimation albeit to a significantly lower degree. These conclusions are supported by the results of semi-empirical AM1/SM2 calculations which predict the hydrogen bonded conformer of type II to be at least 2 kcal/mol less stable than the non-hydrogen bonding ones of type I.

NMR coupling constants are consistent with a predominating ring conformation (>90%) of type ^{4}E or $^4T_3$ characterized by an dihedral angle ω_1 of 120–150 deg [5]. In the present study, the calculated lowest energy conformers obtained by the three force fields all have an approximate $^4T_3$ ring conformation with a calculated dihedral angle ω_1 of 152–154 deg (Table 3) in agreement with experimental data.

Conformational energy penalty for binding of kainate to iGluR2

The conformational energies penalty for binding of kainate to iGluR2 calculated as described in the Computational Methods section and in ref. 11 is 1.9 and −0.2 kcal/mol for AMBER* and MMFF94, respectively. The low conformational energy penalties calculated by these force fields are compatible with the previously reported results of a study of 33 ligand-protein complexes including 28 different ligands. In that work the great majority of the conformational energy penalties was calculated to be less than 3 kcal/mol [11].

Using the lowest energy conformation calculated by MM3*, the conformational energy penalty becomes 43.7 kcal/mol. This unrealistically high energy value is due to the incorrect prediction by this force field of a lowest energy conformer of the intramolecular hydrogen bonded type II in Figure 5. The transformation of this type of conformations into the bioactive conformation of type I requires the breaking of a strong ion-pair hydrogen bond. By using conformer 2 in the MM3* calculations, which is of type I, the calculated conformational energy penalty becomes 2.0 kcal/mol.

Considering the result described above, the lower affinity of kainate for the AMPA receptors compared to the affinity at the KA receptors is most probably not due to a significantly higher conformational energy penalty for binding to AMPA receptors compared to that for binding to KA receptors. A relative affinity of a factor of 600 corresponds to a free energy difference for binding of 4 kcal/mol. Even in the unlikely situation in which kainate binds to KA receptors in exactly the same conformation as the global energy minimum conformation in aqueous solution (i.e., the conformational energy penalty equals zero) the energy penalties calculated by AMBER* and MMFF94 described above would only give an lower affinity at AMPA receptors by a factor of 20 or less.

This conclusion is strongly supported by the recent synthesis and pharmacological testing of (S)-3a,5,6,6a-tetrahydro-4H-pyrrolo[3,4-*d*]isoxazole-3,4-dicarboxylic acid (CIP-AS, Figure 6). This compound displays high affinity for AMPA as well as for KA receptors [2]. In neutral water, CIP-AS has the same

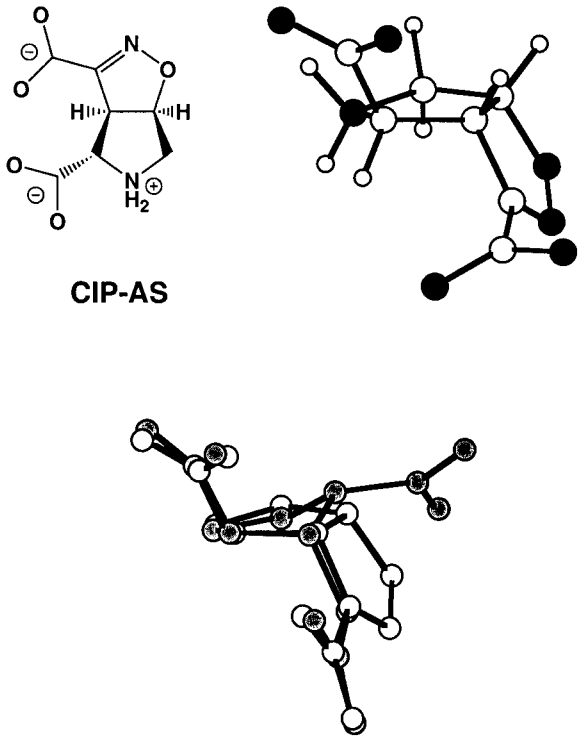


Figure 6. The calculated AM1/SM2 global energy minimum conformation for CIP-AS (top) and a least-squares superimposition of CIP-AS (unfilled atoms) and kainate in its iGluR2-bound conformation (filled atoms). The carboxylate group connected to the isoxazole ring in CIP-AS has been reoriented in order to obtain an optimal fit with the corresponding carboxylate group in kainate.

protonation state as kainate. Due to lack of reliable force field parameters for the isoxazole part of CIP-AS, the conformational analysis of this compound was performed by using AM1/SM2 and AM1/SM5.4. Conformational analysis of the CIP-AS in aqueous solution resulted in a single stable conformation (Figure 6). This conformer displays a gauche (−) conformation with respect to dihedral angle ω_2 (calculated values are −64.9 and −66.0 degrees by AM1/SM2.1 and AM1/SM5.4, respectively). The calculated values for the ω_1 angle are 134.7 and 133.0 deg. A rotation about the bond connecting the ω-carboxylate group to the isoxazole ring by 70 deg increases the energy by 1 kcal/mol. A least-squares superimposition of CIP-AS in this conformation with the experimentally determined iGluR2-bound conformation of kainate is shown in Figure 6. The atoms in the carboxylate groups and the nitrogen atoms were used as fitting points. The two molecules display an excellent fit with an rms value of 0.186 Å. Since CIP-AS binds equally well to AMPA and KA receptors, the superimposition strongly suggests that kainate has very similar bioactive conformations at the AMPA and KA receptors. This supports the conclusion made above that the reason for the lower affinity of kainate at AMPA receptors is not due to a significantly higher conformational energy at these receptors compared to KA receptors. Since CIP-AS in contrast to kainate displays high affinities for AMPA as well as KA receptors, the superimposition in Figure 6 indicates that differential repulsive and/or attractive interactions between the isopropenyl group in kainate and the receptors is the main determinant for the selectivity of kainate for KA receptors.

Conclusions

Conformational analyses of kainate in aqueous solution (GB/SA) by using the MM3*, AMBER* and MMFF94 force fields and a comparison of the calculated results with experimentally determined conformational data indicate that MM3*-GB/SA strongly overestimates the stability of a hydrogen bonded ion-pair in aqueous solution in comparison with the separated ions. This results in an incorrect prediction by MM3* of the most stable conformer of kainate in aqueous solution, whereas AMBER* and MMFF94 correctly predict the lowest energy conformer.

Calculated conformational energy penalties for binding of kainate to the AMPA iGluR2 receptor indicate that the lower affinity of kainate at AMPA receptors compared to KA receptors is not due to a higher energy bioactive conformation of kainate at AMPA receptors. This conclusion is supported by conformational analysis of a nonselective AMPA/KA ligand (CIP-AS) and a comparison of the conformational and structural properties of this ligand with the bioactive conformation of kainate at the iGluR2 receptor. The conclusion of this comparison is that it is highly probable that kainate binds to AMPA and KA receptors in closely the same conformation.

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Appendix

Force field parameters added to the MM3* force field in MacroModel version 6.5 to ensure equality of carboxylate oxygens.

Bending Interactions (BND)

	Angle (deg)	Force const. (mdyn/rad**2)	Bend-Bend FC	Atm1	Atm2	Atm3
C3 - C2 - OM	125.1000	0.8500	0.2400	0000	O200	0000
C3 - C2 = O2	125.1000	0.8500	0.2400	0000	OM00	0000
H1 - C2 - OM	119.2000	0.8500	0.3000	0000	O200	0000
H1 - C2 = O2	119.2000	0.8500	0.3000	0000	OM00	0000
OM - C2 = O2	134.0000	0.8000	0.2400	0000	0000	0000

Torsional Interactions (TOR)

	V1	V2	V3	Atm1	Atm2	Atm3	Atm4
	(V1, V2 & V3 in kcal/mol)						
C3 - C3 - C2 – OM	−0.4000	1.0000	0.0000	0000	0000	O200	0000
C3 - C3 - C2 = O2	−0.4000	1.0000	0.0000	0000	0000	OM00	0000
N2 - C3 - C2 - OM	0.0000	0.8000	1.5000	0000	0000	O200	0000
N2 - C3 - C2 = O2	0.0000	0.8000	1.5000	0000	0000	OM00	0000
00 - C3 - C2 - OM	0.0000	0.0000	0.2000	0000	0000	O200	0000
00 - C3 - C2 = O2	0.0000	0.0000	0.2000	0000	0000	OM00	0000

Torsion-Stretch Interactions (TS)

	FC (kcal/mol)			Atm1	Atm2	Atm3	Atm4
O2 = C2 - C3 - 00	0.1000	0.0000	0.0000	0000	OM00	0000	0000

Out of Plane Bending

	Angle	Const.	Atm1	Atm2	Atm3	Atm4
C2 * OM * 00 * 00	0.0000	0.8000	0000	0000	0000	0000

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