Molecular Recognition of Amiloride Analogs: A Molecular Electrostatic Potential Analysis. 1. Pyrazine Ring Modifications

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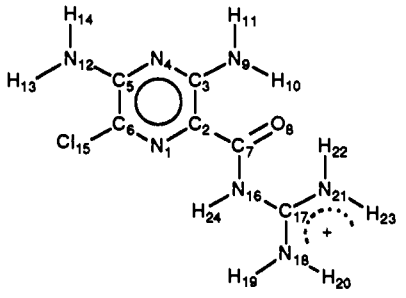
Ab initio molecular electrostatic potential (MEP) patterns are used to determine the electrostatic requirements for the formation of a stable blocking complex between amiloride analogs and the epithelial sodium channel of *Rana ridibunda*. MEP maps calculated in the 3-21G(*) and STO-3G basis sets for amiloride and analogs with pyrazine ring modifications are used to interpret differences in the microscopic rate constants for analog–channel binding determined by Li et al. MEP maps of the protonated analogs are correlated to differences in the value of k_{on}, the microscopic association constant. Those analogs with k_{on} values similar to amiloride are found to have a MEP maximum that is localized over the side chain, as well as strong, distinguishing minima in the MEP pattern off the carbonyl oxygen and positions 3, 4, and 5 of the pyrazine ring. MEP maps of a model-encounter complex (protonated analog and formic acid anion) are correlated to differences in k_{off}, the microscopic dissociation constant. The major conclusions of this work are that (1) a stable blocking complex is formed with analogs which have a deep, localized minimum off the 6 position of the pyrazine ring, (2) the stability of the blocking complex is directly related to the depth of that minimum, (3) substitution at position 5 affects not only the depth but also the location and size of the minimum off position 6, and (4) steric factors may influence the optimal binding of the 6-position ligand to the ion channel. The MEP analysis also suggests that the distance between the proton donors of the chelating guanidinium moiety and the deep, localized minimum off position 6 of the pyrazine ring may define an important spatial requirement for all those analogs which form a stable blocking complex with the channel.

Introduction

Amiloride, 1, a potassium-sparing acylguanidine diuretic, has been shown to be a potent inhibitor of sodium



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transport in a variety of cellular and epithelial transport systems[1] and has been used to probe the mechanism of taste transduction.[2-5] Several recent reviews[1] have summarized the pharmacology of amiloride inhibition of ion transport systems. For example, the protonated form of amiloride (shown as 1) has been shown to inhibit the epithelial sodium channel, the Na^+/H^+ antiporter, and the Na^+/Ca^{2+} exchanger, as well as voltage-gated Na^+ and Ca^{2+} channels, a K^+ channel, the nicotinic acetylcholine receptor, and Na^+/K^+-ATPase. In addition, amiloride inhibits growth factor-induced DNA, RNA, and protein synthesis, and acts at several cellular receptors. Of the ion transport systems, the sodium channel exhibits the highest affinity for amiloride with a K_i' in the submicromolar range. The Na^+/H^+ and Na^+/Ca^{2+} antiporters have K_i' in the micromolar and millimolar ranges, respectively.

Although radioligand binding studies[6] have located the amiloride binding site on sodium channels purified from bovine renal papilla and amphibian cultured A6 cells, no data is yet available on the molecular structure of the sodium channel. In the absence of explicit structural data for such ion transport proteins, structure–activity studies involving amiloride analogs provide a means of probing the steric and electrostatic requirements of the amiloride

Table I. Structure–Activity Relationships for Amiloride Analogs with Pyrazine Ring Modifications[a]

analog	substituent position 5	substituent position 6	k_{on} ($s^{-1}\ \mu M^{-1}$)	k_{off} (s^{-1})	block time (ms)
1	NH_2	Cl	13.17 ± 0.25	3.93 ± 0.19	255
2	NH_2	Br	14.19 ± 1.09	5.58 ± 0.92	179
3	NH_2	I	11.43 ± 0.90	17.41 ± 0.40	57
4	NH_2	F	13.54 ± 0.65	32.20 ± 1.57	31
5	NH_2	H	14.47 ± 0.68	176.25 ± 17.73	6
6	H	Cl	3.32 ± 0.44	10.89 ± 1.35	92
7	Cl	Cl	5.16 ± 0.46	151.10 ± 16.48	7

[a] Data from ref 8.

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binding site. The preferred ground state structure of the free base and protonated forms of the parent compound, amiloride, have been determined by Smith et al.[7] using a combination of ^1H, ^{13}C, and ^{15}N NMR techniques and CNDO/2 theoretical calculations. Reference 1 reviews much of the structure–activity data that correlates modifications of the structure of amiloride to changes in its efficacy as an ion transport inhibitor. In summary, the studies show that amiloride analogs exhibit relatively similar behavior for binding to the Na^+ channel and the Na^+/Ca^{2+} antiporter. Modification of the terminal guanidine nitrogen by substitution with hydrophobic groups enhances the inhibition of the Na^+ channel and the Na^+/Ca^{2+} exchanger, while decreasing inhibition of the Na^+/H^+ antiporter. Substitution of the chlorine at position 6 of the pyrazine ring with hydrogen or fluorine leads to decreased inhibition of all three ion transport systems. In contrast, substitution with bromine or iodine results in decreased inhibition of the Na^+ channel and the Na^+/Ca^{2+} antiporter, but increased inhibition of the Na^+/H^+ antiporter. Alkyl substitution in place of the amino group at position 5 of the pyrazine ring decreases inhibition of the Na^+ channel, but increases inhibition of the two antiporters.

Using a set of 30 amiloride analogs, Li et al.[8,9] have carried out a series of electrophysiological studies on the apical sodium channels of the abdominal skin of *Rana ridibunda*. These experiments relate differences in the microscopic rate constants for the binding of the analog to alterations in the molecular structure of the pyrazine ring[8] or to alterations in the acylguanidinium side chain[9] bonded to the pyrazine ring at position 2. Table I summarizes the results for most of the analogs in the pyrazine ring study. The analogs are identified by the numbering scheme used in ref 8. The structure–activity data in Table I correlate changes in the substituent pattern at positions 5 and 6 of the pyrazine ring to differences in the microscopic association constant, k_{on}, and the dissociation constant, k_{off}. Table I shows that substitution of bromine, iodine, fluorine, or hydrogen for chlorine at position 6 of the pyrazine ring has little effect on k_{on}. However, k_{off} for the series steadily increases to the point that analog 5 forms a significantly less stable blocking complex with the channel than does amiloride. Conversely, substitution of hydrogen (analog 6) or chlorine (analog 7) for the amino group at position 5 of the pyrazine ring affects both k_{on} and k_{off}. For 7, the increase in k_{off}, which implies a less stable blocking complex, is particularly notable.

From these data, Li et al.[8,9] postulated a two-step model for the amiloride–channel interaction. In the first step, the guanidinium side chain of the analog invades the channel entrance and interacts with an anionic site on the channel to form an encounter complex. Then the chlorine atom of amiloride binds to an electropositive site on the channel, leading to the formation of a stable blocking complex. Since the molecular structure of the channel is not known, the specific molecular interactions involved in the amiloride–channel complex have not been further identified.

It is the goal of work in this laboratory to interpret the activity of the amiloride analogs at the molecular level by an analysis of the steric and electrostatic features which determine the optimal interactions of amiloride with the channel binding site. As a first step in that direction, we choose to study the above analogs with pyrazine ring modifications (see Table I). Since these analogs are, in general, sterically similar to amiloride, we are able to focus primarily on the electrostatic features of molecular recognition. It is assumed in this analysis that (1) the channel binding site is complementary in both shape and electrostatics to the analogs and (2) that the most stable complex is formed by that analog, i.e. 1, which exhibits the best steric and electrostatic fit to the binding site. In a future publication, we will analyze further the steric and electrostatic components of molecular recognition through a study of amiloride analogs with elongated side chains.

The electrostatic features of molecular recognition are determined by comparison of ab initio molecular electrostatic potential (MEP) patterns of the amiloride analogs. In this way, we are able to relate differences in the MEP patterns of the analogs to differences in the kinetic data for formation of the encounter and blocking complexes. In addition, we develop a hypothesis describing the important molecular recognition features which determine the stability of the blocking complex. We have used a similar approach to interpret structure–activity data on the sweet-taste potencies of a series of perillartine and nitroaniline analogs[10–13] and acesulfame.[14] The computational protocol is described in the Methodology section below.

Methodology

Selection of Analogs. Of the 10 analogs studied by Li et al.,[8] the seven listed in Table I were selected for the computational study. The three remaining analogs were not used because, although it was demonstrated[8] that they are high-rate blockers competitive with amiloride, the spectra of the analogs did not show a clear Lorentzian

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component in the required frequency range and, as a result, k_{on} and k_{off} could not be determined.[8]

Geometry Optimization. Due to the large size of the analogs, full geometry optimization was not carried out. The optimized geometry of the pyrazine ring and guanidinium side chain were taken from our molecular orbital study of amiloride in the 3-21G(*) basis set.[15] Then, the ring and side chain were connected to form the complete molecule as described previously.[15] Comparison of the MEP of the free base form of 1 calculated in this geometry to that calculated in the fully-optimized geometry showed no obvious difference in the MEP.

Analogs 2 and 3 were formed from 1 by adding bromine and iodine at bond lengths of 1.85 Å and 2.05 Å,[16] respectively. Analogs 5–7 were formed from 1 by adding hydrogen or chlorine at positions 5 or 6 with bond lengths of 1.08 Å and 1.74 Å,[15] respectively. For 4, because of potential hydrogen-bonding interactions between the fluorine substituent at position 6 and the amino group at position 5, the bond angles and bond lengths involving these substituents were optimized using the 3-21G(*) basis set for analog 4. As a result, the H_{13}–N_{12}–C_5, N_{12}–C_5–C_6, and C_5–C_6–F_{15} angles of 4 all decreased by 1–2° compared to those of amiloride, bringing H_{13} 0.14 Å closer to fluorine in 4 than to chlorine in 1. The optimized hydrogen–fluorine bond distance was 1.34 Å.

Our 3-21G(*) torsional barrier study of the free base form of 1[15] had indicated that the amiloride conformer with O_8–C_7–C_2–N_1 = 180° was the conformer of lowest energy and that a large barrier of 19 kcal/mol was associated with torsion around this dihedral angle. Since substitution at positions 5 or 6 of the pyrazine ring should not significantly affect the height of this barrier, all the analogs in this study were assumed to adopt the conformation with O_8–C_7–C_2–N_1 = 180°.

Molecular Electrostatic Potential Patterns. Although the formation of the blocking complex may be a concerted procedure, in order to approach the problem computationally, we used a stepwise protocol. (1) The MEP patterns of the unprotonated analogs were calculated and inspected in order to determine the site of protonation. (2) Then, the protonated forms of the analogs were constructed and their MEPs were calculated. These were inspected and compared to differences in the value of k_{on} in order to identify features in the MEP pattern of the analog that might be related to the formation of the encounter complex. (3) Finally, the MEP maps of a simple model encounter complex (protonated analog and formic acid anion) were calculated and compared to differences in k_{off} in order to identify features in the MEP pattern of the analog that might be related to the formation of a stable blocking complex. The model encounter complex was not meant to model the complete analog–channel interaction. Rather, it was used to illustrate the change in the MEP pattern of the analog that occurs upon binding to a putative anionic site and to thereby highlight the molecular recognition features of the bound analog that might be implicated in the formation of a stable blocking complex.

The MEP patterns of the free base and protonated forms of the analogs were calculated in the 3-21G(*) basis set. Due to computer disk space limitations, the MEP maps of the model encounter complexes were calculated in the STO-3G basis set. Since only STO-3G but not 3-21G(*) basis function parameters are available for bromine and iodine, the MEP patterns of only the model encounter complexes were calculated for 2 and 3. Comparison of the 3-21G(*) and STO-3G maps of the free base form of amiloride showed that the STO-3G map was able to reproduce the general location although not the depth of the minima noted in the 3-21G(*) maps. For example, the STO-3G basis set showed the minimum off the oxygen to be less negative and the minimum off the chlorine atom to be more distinct than the respective 3-21G(*) minima. However, allowing for these small differences, this procedure still makes possible a qualitative identification of important molecular recognition features.

All the maps were calculated in a plane 1.3 Å from the plane of the molecule using the GAUSSIAN86[17] program. In addition, since chlorine, bromine, and iodine have larger van der Waals radii[18] than fluorine (1.80 Å, 1.95 Å, and 2.15 Å versus 1.35 Å, respectively), MEP maps of the model encounter complex with 1, 2, and 3 were also calculated in a plane 1.8 Å from the plane of the molecule. The maps were displayed using the ARCHEM[19] program. All calculations were carried out on the VAX8800 and VAX6430 at the New Jersey Institute of Technology.

Model Encounter Complex. The geometry of the formic acid anion was optimized in the 3-21G(*) basis set. Then, the model encounter complex was constructed with the oxygen atoms of the formate in a chelate-type orientation with respect to the guanidinium group. This orientation was chosen because it has been noted as the most common orientation of the carboxylate moiety and the arginine residue in crystal structures of proteins and peptides.[20–24] The CHEM-X[25] molecular modeling system was used to move the formate moiety into the same plane as the analog and to set the H (analog)···O (formate) distance to 1.8 Å. This distance is slightly larger than the H···O distance of 1.6 Å obtained by ab initio geometry optimization of related guanidinium–carboxylate systems.[26–28]

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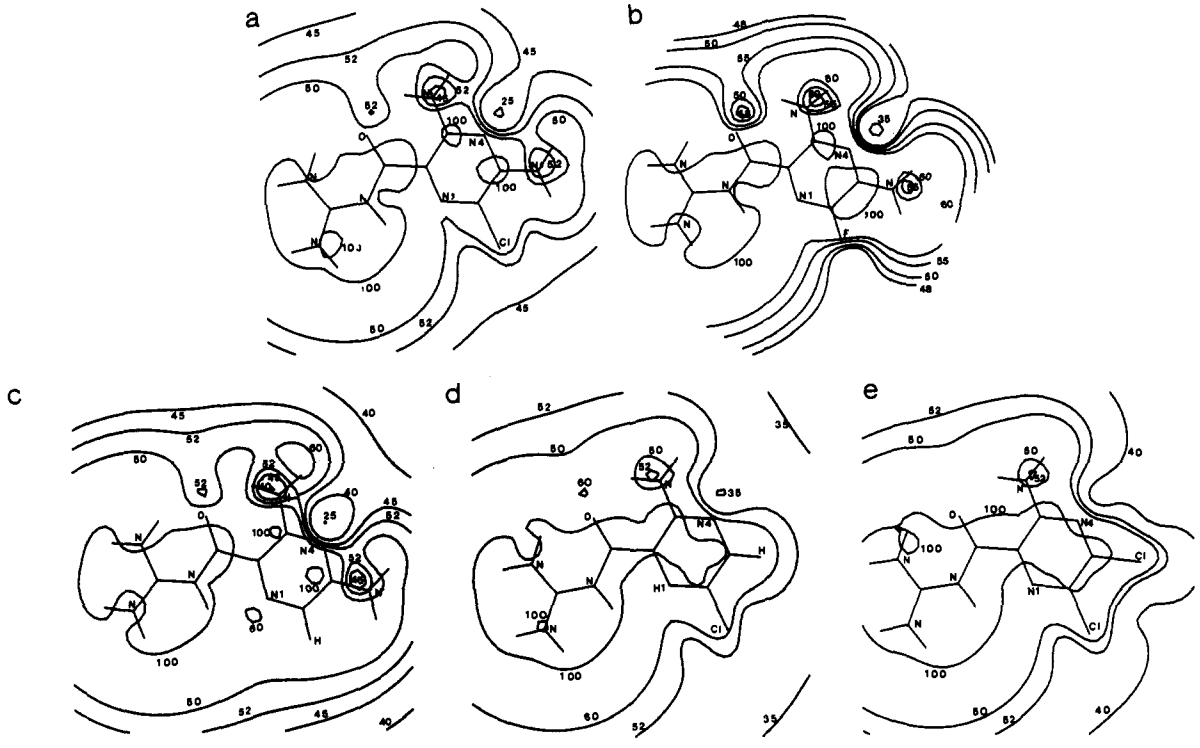

Figure 1. The molecular electrostatic potential pattern of the amiloride analogs: (a) 1, (b) 4, (c) 5, (d) 6, (e) 7. Contour levels are given in units of kcal/mol.

Results

The Site of Protonation. The site of protonation was determined as the atom which showed the deepest MEP minima in the maps of the free base forms of the analogs. In our previous work,[15] we had shown by MEP analysis that the imino nitrogen, N_{16}, is the site of protonation of 1. The MEP maps of the free base forms of 5, 6, and 7 (not shown) are similar to that of 1 and predict the imino nitrogen as the site of protonation. The imino nitrogen was assumed to be the site of protonation for analogs 2, 3, and 4, as well.

Molecular Electrostatic Potentials of the Protonated Analogs. Figure 1 gives the MEP maps of the protonated analogs and is used to determine which molecular recognition features of the analogs are involved in the formation of the encounter complex. All the maps exhibit a broad maximum in the MEP over the guanidinium side chain and show that the positive potential is spread out over the molecule rather than being localized near the amino groups, as the 1+ charge is often depicted in schematic diagrams of amiloride like 1. Figure 1a shows that the MEP of protonated amiloride has local minima in the positive potential off the carbonyl oxygen, the amino groups at positions 3 and 5 of the pyrazine ring, and at N_4 of the pyrazine ring, the location of the global minimum. The map also exhibits a local minimum off the chlorine atom. Figure 1b shows the MEP map of 4, in which fluorine has replaced chlorine at position 6 of the pyrazine ring. The positions of the local minima are the same as for 1, with the exception that the minimum off position 6 no longer stretches toward N_1 in 4. Since fluorine is more electronegative than chlorine, the positive maximum of 100 kcal/mol extends over more of the pyrazine ring in 4 than 1. Figure 1c shows the MEP map of 5, in which hydrogen has replaced chlorine at position 6 of the pyrazine ring. The positions of the local minima are the same as for 1, with the exception of the minimum off position 6, which is missing in 5. The spatial extent of the positive maximum over the guanidinium group is similar in the three analogs 1, 4, and 5.

Figure 1d shows the MEP map of 6. The replacement of the electron-donating amino group of 1 by a hydrogen to form 6 has significantly altered the MEP pattern of the molecule. Neither the carbonyl oxygen nor positions 3, 4, and 5 of the pyrazine ring show minima of the depth of 1 and 5. In addition, the broad, positive maximum over the guanidinium side chain extends over the ring of 6. Figure 1e shows the MEP map of 7 which has a double substitution of chlorine at positions 5 and 6. As with 6, the map of 7 has lost the four distinguishing minima off the carbonyl oxygen and off positions 3, 4, and 5 of the pyrazine ring. Again, the broad, positive maximum over the guanidinium side chain of 7 extends over the ring, as in 6.

Molecular Electrostatic Potentials of the Model Encounter Complexes. Figure 2 shows the MEPs of the model encounter complexes and is used to determine which molecular recognition features are important in the formation of a stable blocking complex. Figure 2a shows the MEP pattern of the model encounter complex formed by 1 and the formate ion. The region around the anion is negative, while most of the region around 1 is positive. However, two negative minima are associated with the pyrazine ring: the minimum off N_4 and the minimum off the chlorine atom at position 6 of the pyrazine ring. Figure 2b–e shows the MEP of the model encounter complexes with 2, 3, 4, and 5, respectively. All of the figures show a pattern very similar to that of 1, with the exception that there is no negative minimum off the atom at position 6. The replacement of chlorine by hydrogen (analog 5) leads to the most significant change in this part of the MEP pattern. The region off position 6 in 5 shows MEP values

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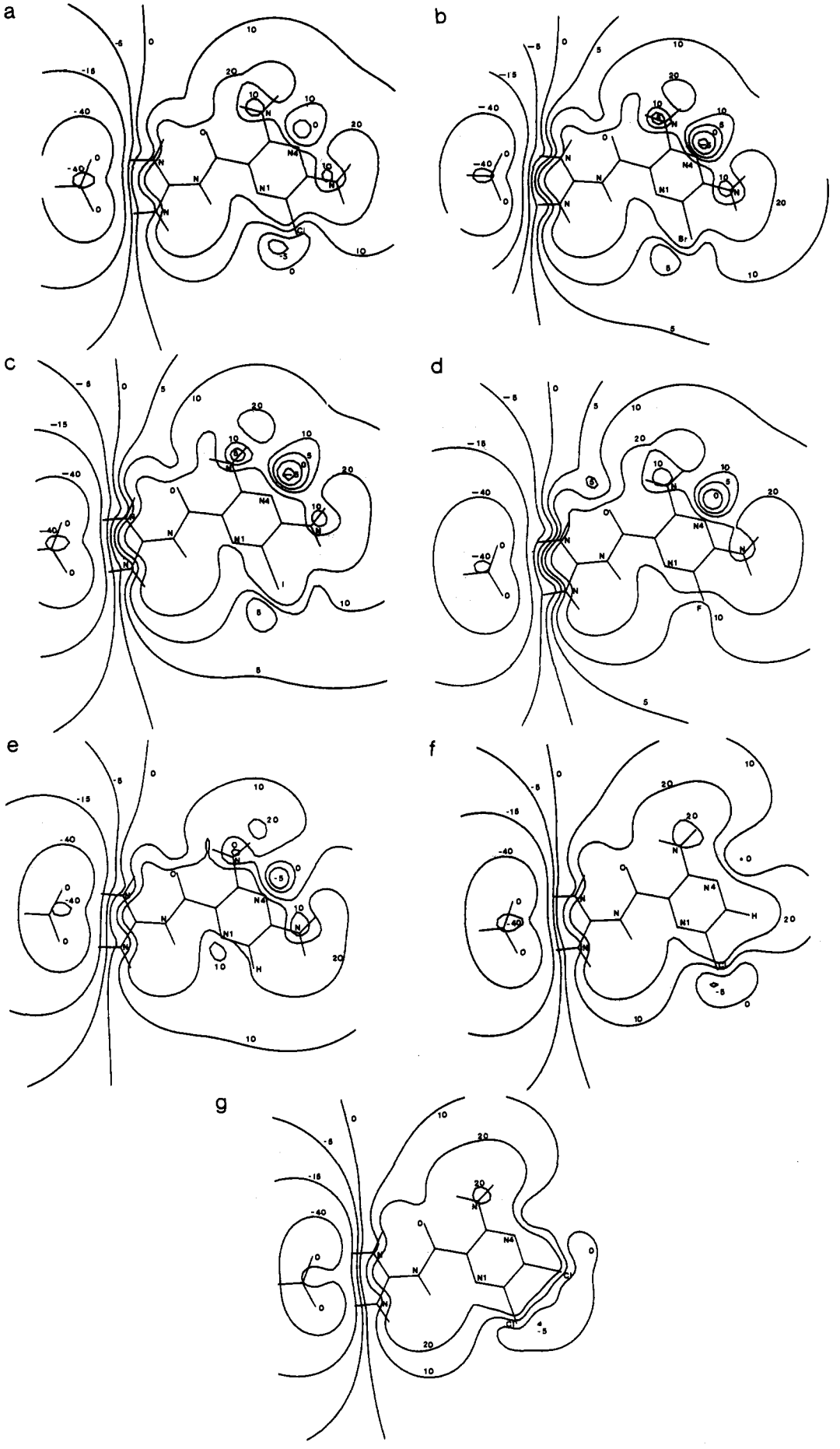

Figure 2. The molecular electrostatic potential pattern of the model encounter complexes formed by amiloride analogs and the formic acid anion: (a) **1**, (b) **2**, (c) **3**, (d) **4**, (e) **5**, (f) **6**, (g) **7**. Contour levels are given in units of kcal/mol. Note that parts b–d have been drawn with an additional contour level at 5 kcal/mol.

of 10–20 kcal/mol in contrast to 1, which shows an MEP value of –5 kcal/mol off the chlorine atom. The MEPs of 2–4 were drawn with an extra contour at 5 kcal/mol in order to further investigate the region off the ligand at position 6. Figure 2b,c show that 2 and 3 have local minima of 5 kcal/mol off the halogen at position 6. Figure 2d, in contrast, shows no such feature off the fluorine. The region off the fluorine is more positive than off the other halogens, possibly due to strong attractive interactions between the fluorine and the neighboring amino group at position 5. Only 5, with a hydrogen at position 6, shows a more positive MEP pattern in this region than does 4. The MEPs of 1, 2, and 3 at 1.8 Å from the plane of the molecule (not shown) exhibit the same relative features as described above. In this case, 2 and 3 show local minima of 5 kcal/mol off position 6, while 1 shows a local minimum of 0 kcal/mol in this region.

For the analogs with substituents at position 5, Figure 2f shows that, in the MEP of the model encounter complex with 6, the minimum off N_4 is missing, but the minimum off position 6 is similar to that of 1. Figure 2g shows the MEP of the model encounter complex with 7. Due to the presence of chlorine atoms at both positions 5 and 6 in 7, the negative region off position 6 is spread out over a large region around positions 5 and 6. The global minimum in the MEP is relocated to a position between the two chlorines, in contrast to the MEP of 1, where it is located between the chlorine and N_1. For 6 and 7, the minima off the N_4 are much less distinctive than for 1–5. All the model encounter complexes exhibit a broad positive region extending from the guanidinium side chain to cover the ring.

Discussion

The Encounter Complex. In comparing the MEP maps to the k_{on} values in Table I, it can be seen that 1, 4, and 5 have similar values of k_{on} for the formation of the encounter complex and have very similar maps, except in the region of position 6 of the pyrazine ring. Since k_{on} is an indication of the initial molecular interactions between the analog and the channel, this seems to indicate that position 6 of the pyrazine ring is not involved in the formation of the encounter complex. Rather, the positively charged side chain most likely initiates the electrostatic interaction with the channel. Since 1, 4, and 5 have very similar minima off the carbonyl oxygen and positions 3, 4, and 5 of the pyrazine ring (see Figure 1a–c), it may be that the functional groups at these positions also interact with the channel to form the encounter complex. Or, alternatively, they may function to control the electrostatic features over the ring which are complementary to those of the channel binding site. In either case, it is clear that position 6 is not implicated in the formation of the encounter complex.

This hypothesis is supported by the maps of 6 and 7 (Figure 1, parts d and e, respectively). Although analog 6 has a local minimum off position 6 as does 1, it has a very different value of k_{on}: 3.32 versus 13.17 s^{-1} μM^{-1} for 1. On the other hand, with 6, the minimum off position 5 no longer exists and those off positions 3 and 4 and the carbonyl oxygen are less deep than those of 1. The same result is seen with 7. Analog 7 has a much smaller value for k_{on} than 1, 4, or 5, and the MEP map of 7 has lost the four distinguishing minima off the carbonyl oxygen and off positions 3, 4, and 5 of the pyrazine ring that were typical of 1, 4, and 5. In addition, for both 6 and 7, the positive maximum of the side chain extends over the ring, resulting in a very different pattern than the MEPs of 1, 4, and 5.

In summary, those analogs that have k_{on} values similar to amiloride have strong, distinguishing minima in the MEP pattern off the carbonyl oxygen, N_4, and the amino groups at positions 3 and 5 of the pyrazine ring. Analogs which have k_{on} values which differ from amiloride lack two or more of these features and exhibit a much more positive pattern on the pyrazine ring. The utility of the MEP method is in identifying electrostatic recognition features of the analogs that can be correlated with values of k_{on}. The method cannot distinguish whether the carbonyl oxygen and pyrazine ring substituents actually interact with the ion channel directly to form the encounter complex or if they in some way control the interaction in a more global fashion by their effect on the overall molecular electrostatic potential pattern of the analog. This latter effect may be important, for example, to the formation of a stacking complex with the channel.

The Blocking Complex. If, as hypothesized,[8,9] the chlorine atom of amiloride interacts with a site on the channel, then the MEP pattern of amiloride in Figure 2a defines the electrostatic requirements for formation of a stable analog–channel blocking complex. Table I shows that the values for k_{off} for 1 and 5 are 3.93 and 176.25 s^{-1}, respectively. Since the residence time for the analog on the channel is inversely proportional to k_{off}, this indicates that 5 forms a very poor blocking complex compared to amiloride. Table I shows that 5 has a block time of only 6 ms compared to 255 ms for 1. Comparison of Figure 2, parts a and e, shows that both molecules exhibit minima off N_4, but only amiloride has an additional minimum off the chlorine atom at position 6 of the pyrazine ring. Therefore, electrostatic interactions between the channel and position 6, rather than position 4, are more important to the stability of the blocking complex. In the case of 5, which exhibits a broad, positive region off position 6 rather than the localized minimum of 1, this electrostatic molecular recognition feature is missing, leading to a poor interaction with a complementary site on the channel.

The MEPs of 2, 3, and 4 taken together with those of 1 and 5 show that the efficacy of an analog as a sodium transport blocker is related to the depth of the minimum off position 6. As the minimum becomes progressively less negative, the block time becomes shorter. This supports the hypothesis of Li et al.[8,9] that the ligand at the 6 position binds to an electropositive site on the channel. Li et al.[8] also noted that formation of a long-lived blocking complex requires a highly electronegative substituent at position 6. But they did not address the fact that, although fluorine is the most electronegative atom in the series, 4 is not the best blocker. The MEP maps, however, clarify this point. In contrast to the deep minimum off the chlorine in 1, 4 has a very positive MEP pattern in this region. This is probably due to the strong hydrogen-bonding interaction between fluorine and the amino group at position 5 noted in the geometry optimization of 4. Therefore, the most electronegative atom does not exhibit the deepest MEP minimum off position 6.

In addition, comparison of the MEP maps of 2 and 3 shows that they are very similar and yet the values of k_{off} are slightly different: 5.58 s^{-1} for 2 and 17.41 s^{-1} for 3. Since iodine has a much larger van der Waals radius than bromine, this suggests that steric factors also influence the binding of the 6-position ligand to the ion channel.

It should be emphasized that, as mentioned in the Methodology section, the depths of the minima are dependent on the basis set used to calculate the MEP. Therefore, what is important in the comparison of maps calculated with a single basis set is not the sign of the

minima (negative in the case of chlorine, positive in the case of bromine), but rather the relative trends within a series of substituents.

The importance of a deep, localized minimum off position 6 is further supported by the model encounter complex with 6 (see Figure 2f). Although the minimum off N_4 is negligible in this complex, the minimum off the chlorine at position 6 is similar to that of 1. Since the k_{off} of 6 (10.89 s^{-1}) is similar to that of amiloride, the presence of this minimum at position 6 seems to be the determining feature in the formation of a stable blocking complex. Further support for this hypothesis is shown by the results for 7. The MEP of 7 (Figure 1e) and the low value of k_{on} for 7 indicate that it does not interact with the channel in the same way as 1 to form the encounter complex. Table I shows that the value for k_{off} for 7 is 151.10 s^{-1}. This indicates that 7 forms a much less stable blocking complex with a block time of only 7 ms compared to 1. Figure 2g shows that the negative MEP pattern off position 6 in 7 is not localized to the same region of space as that in 1. Rather, due to the presence of two chlorine substituents, it is spread out in a broad region around positions 5 and 6, with the global minimum located between the two positions.

Li et al.[8] have suggested that the electron-donating amino group at position 5 of 1 stabilizes the complex by increasing the electron density of the ligand at the 6 position relative to 6 and 7. The MEP analysis further elucidates this explanation by illustrating the specific effects of substitution at position 5. The decrease in the depth of the minima at position 6 indicates how the electron density at this position has been changed by the substitution of hydrogen or chlorine for the amino group. However, the MEP analysis further shows that, in the case of 7, the consequence of substitution is that the global minimum has also been shifted to a different location than in 1. Since the region off position 5 is positive in 1 and 6, which form stable blocking complexes, but negative in 7, it is possible that the broad negative MEP in 7 may lead to unfavorable electrostatic interactions with the channel, and ultimately to a poor blocking complex. So, in summary, the effect of the substitution of chlorine at position 5 may be not only to relocate the position of the MEP minimum but also to lead to additional unfavorable electrostatic interactions with the channel.

Conclusions

Molecular electrostatic potential patterns of amiloride analogs have been used to interpret the molecular electrostatic recognition features which regulate the formation of the initial encounter complex and the stable blocking complex. This study has assumed that amiloride binds to a site which is complementary to itself in both steric and electrostatic features. As part of this approach, it is therefore reasonable to assume, as postulated by Li et al.,[8,9] that the charged guanidinium moiety is neutralized by interaction with a carboxylate group found in an Asp or Glu residue of a channel protein. Therefore, the strong electrostatic interaction between the carboxylate and guanidinium groups will dominate the initial molecular interactions between the analog and the channel by orienting and directing the analog into the binding site. Then, more specific electrostatic and steric effects will come into play.

The structure–activity studies of Li et al.[8] identified the presence of the chlorine substituent at position 6 to be crucial to the formation of a stable blocking complex. The molecular orbital approach described here has built upon this work by defining in greater detail the specific steric and electrostatic requirements of the amiloride binding site. The utility of the MEP approach has been to identify the molecular electrostatic recognition features which regulate the formation of the encounter and blocking complexes. The major conclusions of this work are that (1) a stable blocking complex is formed with analogs which have a deep, localized minimum off the 6 position of the pyrazine ring, (2) the stability of the blocking complex is directly related to the depth of that minimum, (3) substitution at position 5 affects not only the depth but the location and size of the minimum off position 6, and (4) steric factors may influence the optimal binding of the 6-position ligand to the ion channel.

The calculations were carried out in vacuo and, therefore, do not include the effects of solvent or protein environment on the MEP. It has been assumed that these effects would be constant for the series of analogs studied here and that inclusion of these damping factors would not affect the general conclusions of the paper. This work has assumed that the analogs adopt a planar orientation with O_8–C_7–C_2–N_1 = 180°. Our studies of the O_8–C_7–C_2–N_1 torsional barrier in 1[29] indicate that there is a large energy penalty for nonplanar orientations. We have also studied the effect of solvent on the conformations of the free base and protonated forms of amiloride through molecular dynamics simulation.[30] We find that solvent does not significantly disrupt the intramolecular hydrogen-bonding pattern shown in 1. Therefore, it is reasonable to assume a planar conformation for the analogs in this study.

Although this work has focused on the electrostatic component of molecular recognition, steric interactions are also important. The binding site must be complementary to both the molecular shape and the molecular electrostatic potential of the analog. The present work suggests that a similar distance between the proton donors of the chelating guanidinium moiety and the localized negative minimum off position 6 of the pyrazine ring seen in the encounter complex of 1 should be found in all those analogs which form a stable blocking complex with the channel. In order to test this steric hypothesis as well as our hypothesis regarding the important electrostatic components of molecular recognition, we are studying a series of amiloride analogs with side-chain modifications.[9] Some of these molecules have been elongated by the insertion of –NH– or –O–, for example, into the acylguanidinium side chain. In our study, to be reported in a future publication, we interpret the kinetic rate constants of the modified side-chain analogs in terms of the steric and electrostatic molecular recognition features proposed here. In this work, alternative modes of carboxylate–guanidinium binding, as well as nonplanar side-chain conformations, are considered.

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(29) Venanzi, C. A.; Skawinski, W. J.; Venanzi, T. J., unpublished results.

(30) Buono, R. A.; Venanzi, C. A.; Venanzi, T. J., unpublished results.