A THEORETICAL STUDY OF Na^+ AND Mg^{+2} BINDING TO THE CARBONYL OXYGEN OF *N*-METHYL ACETAMIDE

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ABSTRACT Molecular orbital calculations (CNDO/2) are reported for the interaction of Na^+ and Mg^{+2} with the carbonyl of a model peptide moiety (*N*-methyl acetamide) as a function of the $\rangle C{-}O \cdots Me$ distance and angle and with variation in the number of ligands for the purpose of determining the steepness of the distance dependence of the binding energy and for the purpose of determining the reduction of charge on the ion with increasing numbers of ligands.

The greater energy derived on divalent ion binding and the steeper distance dependence indicate that selective, divalent over monovalent, ion binding will occur whenever the liganding system can provide a coordination shell of appropriate dimension. The calculations indicate that the preferred $C{-}O \cdots Me$ angle is not 180°. Of particular note is the decrease of charge on the cation on binding to *N*-methyl acetamide. One ligand bound to Na^+ reduces the charge from 1.0 to 0.7 electron units and four ligands bound to Mg^{+2} reduces the charge from 2.0 to 0.7 electron units. This is of primary significance in carrier and channel mechanisms for cation permeation of lipid membranes; and although the numerical values are qualitative, the implication is for allowance of multiple occupancy of channels by monovalent cations.

INTRODUCTION

Theoretical treatments of metal-ligand interactions have received considerable attention in recent times (1–3) due their importance in the function of metal-containing biological molecules (4, 5). Recent advances in *ab initio* calculations and the availability of computer routines have resulted in some *ab initio* studies of the alkali and alkaline earth metal complexes (6–10). Only recently, an *ab initio* study of the Ca^{+2} interaction with $H_2PO_4^-$ and $(CH_3O)_2PO_2^-$ has been reported in the literature (8). However, a number of semiempirical calculations of the alkali and alkaline earth metal complexes with nucleic acid bases, depsipeptides and peptides have appeared in the literature (11, 12). Such calculations have utilized both extended Hückel theory (EHT), the complete neglect of differential overlap approximation (CNDO/2) and molecular orbital approximations (13, 14). Semiempirical calculations provide a qualitative insight into the nature of metal-ligand bonding. In the present study

the interactions of Na^+ and Mg^{+2} cations with the C=O group of *N*-methyl acetamide, a model for the peptide moiety, have been investigated using the CNDO/2 molecular orbital calculations. The carbonyl oxygen is implicated in cation transport across lipid membranes, is involved in selective binding of alkali and alkaline earth cations to proteins and polypeptides, and has been suggested by Urry (15, 16) as the primary ligand for selective Ca^{+2} binding to elastin and possibly collagen.

In this report attention was directed toward Na^+ and Mg^{+2} binding to the peptide carbonyl group. It is hoped that the model system chosen above will provide some initial insight into the binding of a univalent cation, i.e., Na^+, and a divalent cation, i.e. Mg^{2+}, to peptide backbone carbonyl oxygens and that the results of such a study may be related to the interactions occurring in more complex, real situations in biological systems. Of fundamental concern is the mechanism of reduction of ion self-energy for permeation of a membrane lipid dielectric barrier.

Molecular orbital theory is capable of treating the atomic orbitals of the cation and the ligand in a single calculational scheme with the potential to answer some aspects of the physiochemical selectivity manifested by different cations (2). On the other hand, a simple point charge approximation is inherently ill-equipped to answer the question of cation selectivity. A point charge scheme fails to differentiate between the electronic structure of different cations. The point charge approximation also assumes that the metal-ligand interactions are electrostatic in nature, whereas it has been shown recently by Pullman (7) that, although the direct electrostatic term is large in proportion to other contributions, the polarization and charge transfer contributions are also significant.

As the cation approaches the C=O group of a peptide moiety with formation of the metal-oxygen bond, there is an appreciable charge delocalization, causing a reduction in the charge on the cation. Such a charge delocalization in the cation-peptide complex has been recently analyzed in connection with the problem of multivalent ions permeating through lipid membranes (16). Calculations were performed for Mg^{+2} surrounded by two and by four *N*-methyl acetamide molecules in order to determine the charge localized on Mg^{+2} in the presence of these ligand fields. Due to computational limitations, higher coordination numbers were not considered (4).

Both Na^+ and Mg^{+2} are involved in biological transport processes and hence it is of interest to learn the details of their interactions with the peptide moiety (17). The objectives of the study have been to investigate the following aspects of Na^+- and Mg^{+2}-peptide carbonyl interactions: (*a*) The distance and angular dependence of the Na^+ and Mg^{+2} interactions with *N*-methyl acetamide, a model system for the peptide moiety, for the purpose of understanding monovalent vs. divalent ion selectivity. (*b*) The delocalization of electronic charge between the cations and the ligand for the purpose of understanding permeation of the dielectric barrier of lipid membranes.

METHODS AND RESULTS

Molecular orbital calculations were performed using the CNDO/2 molecular orbital approximation, extensively discussed elsewhere (14), and have been earlier applied to metal-ligand inter-

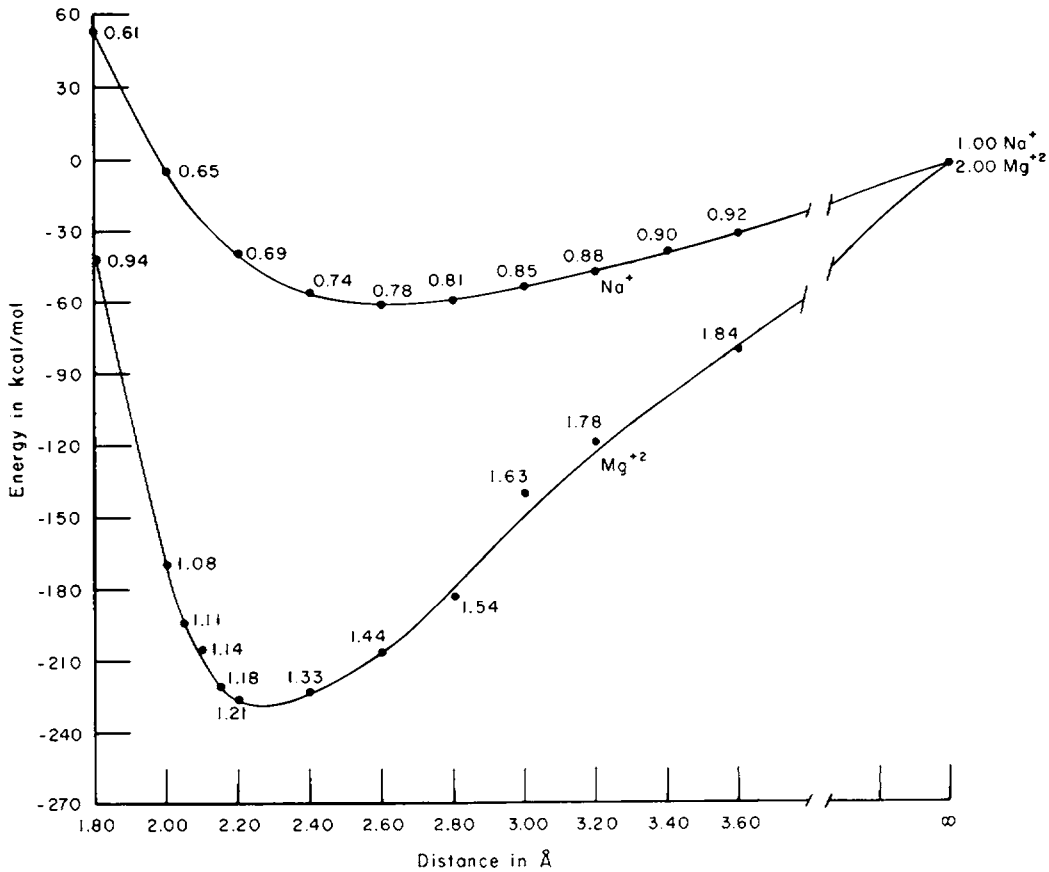

FIGURE 1 CNDO/2 total energy in kcal/mol of the supermolecular containing the cation i.e. Na^+ or Mg^{+2} as a function of the Me $\cdots$ O distance in angstroms. Electronic charge on the cation is marked on the respective curves.

actions (11). The basis set considered was a minimal basis set with 1s functions for H; 2s, 2p functions for C, N, and O, and 3s, 3p, 3d functions for Na^+ and Mg^{+2} cations. Slater orbitals were used for the calculation of overlap integrals with exponents taken from Santry and Segal (18). Parameters, $1/2\ (I_s + A_s)$; $1/2\ (I_p + A_p)$; $1/2\ (I_d + A_d)$, where Is and As are ionization potentials and electron affinities, respectively; β_A^0 for Na^+ and Mg^{+2} were taken from Santry and Segal (18) with no modifications. Standard geometry was used for *N*-methyl acetamide (19). Methyl hydrogen atoms were assumed to be in a staggered conformation. Initially the Na^+ cation was taken to approach the *N*-methyl acetamide along the C'—O axis, i.e., with the $Na^+ \cdots O{=}C'$ angle being 180°. The total energy of the super-molecule was calculated for different $Na^+ \cdots O$ distances, ranging from 1.8 Å to 3.6 Å at 0.2 Å intervals. The electronic charge on Na^+ as a function of distance was also calculated. The results are presented in Fig. 1. The electronic charge in electron units for all atoms in the Na^+-*N*-methyl acetamide complex, in the case of the calculated optimal $Na^+ \cdots O$ distance of 2.6 Å, is shown in Fig. 2a.

Using the optimal $Na \cdots O$ distance of 2.6 Å, we performed a clockwise and counterclockwise rotation of the ligand about the *z* axis, i.e. perpendicular to the plane containing

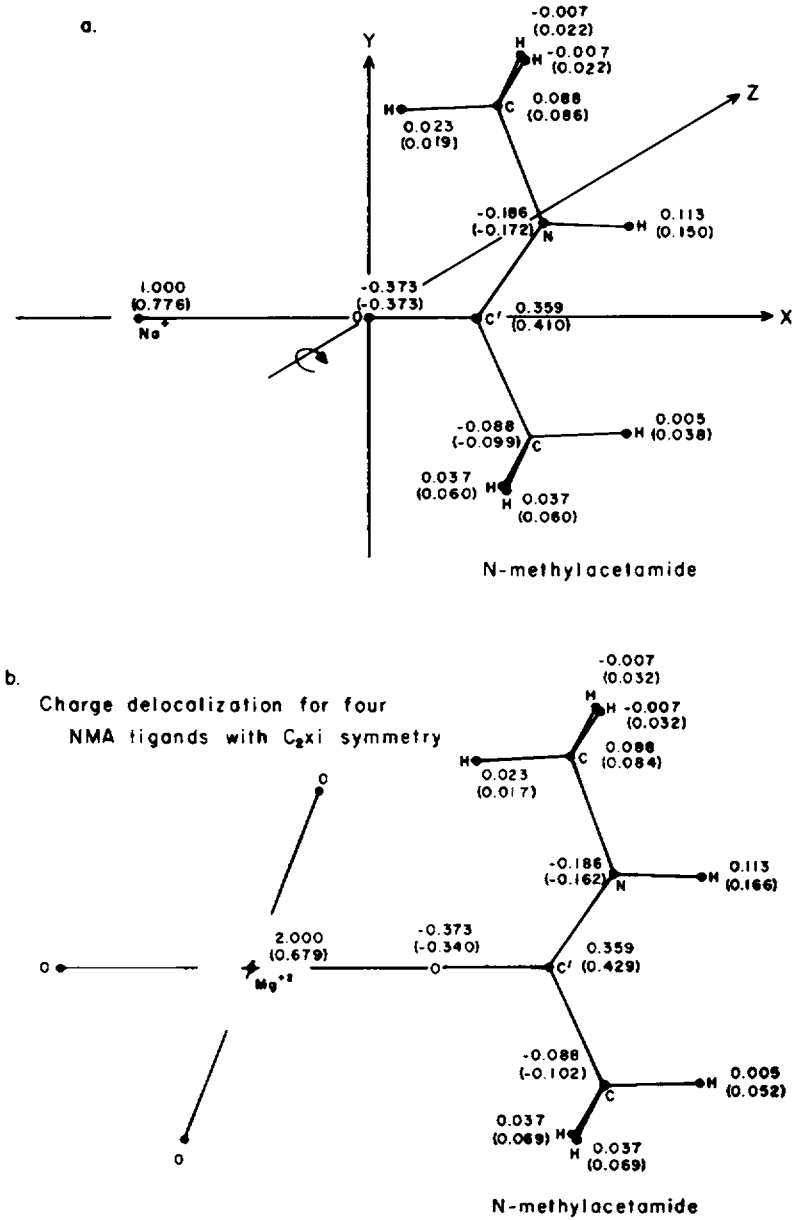

FIGURE 2 (*a*) Net electronic charges in electron units for the Na^+ *N*-methyl acetamide complex. The Na · · · O distance was taken at the calculated minimum energy distance of 2.6 Å. The *x*, *y*, and *z* axes are defined. The rotation was performed about the *z* axis in generating the plot in Fig. 3 below. (*b*) Net electronic charges in electron units for the Mg^{+2} (*N*-methyl acetamide)$_4$ complex.

the cation and *N*-methyl acetamide backbone atoms (see Fig. 2*a*) for definition of the axes). The rotation either increased or decreased the interatomic distance between the cation and the nitrogen atom in the ligand, representing situations where the cation was closer to the nitrogen or α-carbon atom of the ligand. The CNDO/2 total energy of the supermolecular complex was calculated at 10° intervals from 0° to 60° when the cation approached the ligand on the nitrogen side, whereas a range of 10°–90° was explored for the cationic approach to the carbon atom side. The results of the above calculations are presented in Fig. 3. The electronic charge on Na as a function of the angle of rotation of the ligand, θ, is also shown in Fig. 3.

Interaction of Mg^{+2} with two molecules of *N*-methyl acetamide, sandwiching the cation, was investigated first for the distance dependence (see Fig. 1) and then for the angular dependence by rotating both the ligands at the oxygen atom as pivots in a clockwise and counterclockwise direction, as described earlier for Na^+ interaction (see Fig. 3). The electronic charges for the Mg^{+2}-(*N*-methyl acetamide)$_4$ complex were calculated using the optimal distance of 2.2 Å for the Mg ··· O distance. The complex was taken to have C_2 *xi* symmetry.

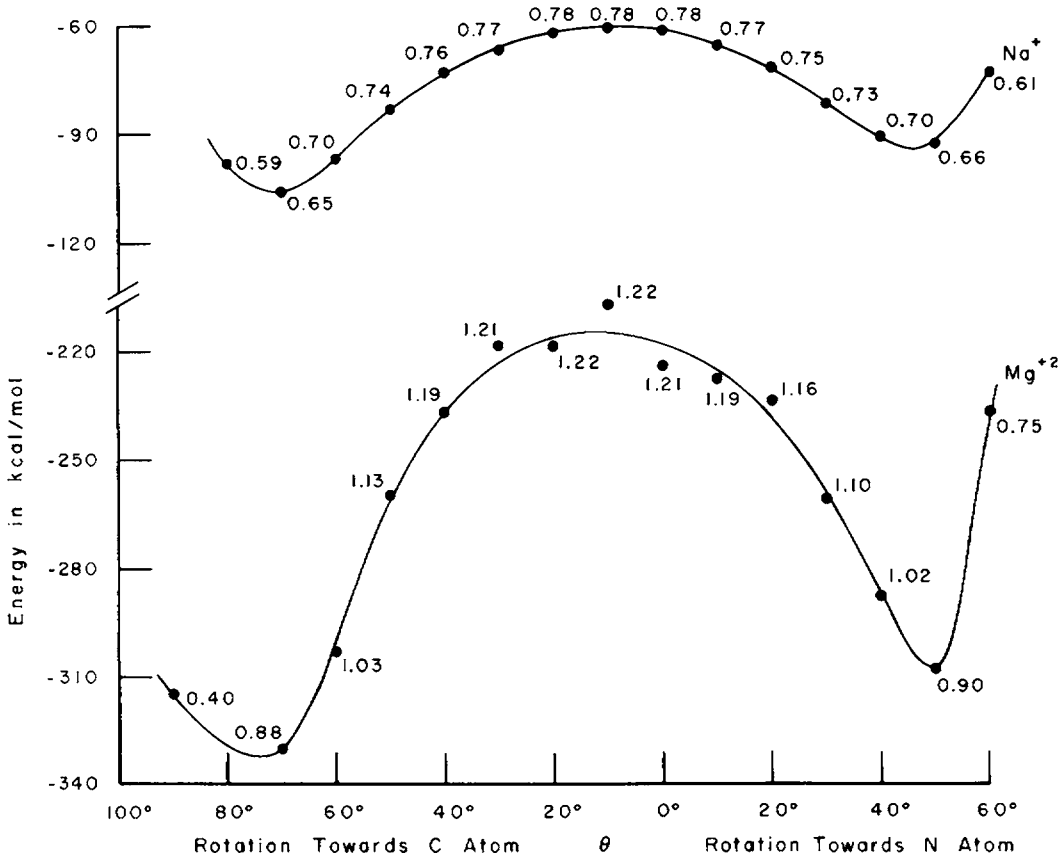

FIGURE 3 CNDO/2 total energy in kcal/mol as a function of rotation angle θ about the *z* axis perpendicular to the plane containing all atoms in *N*-methyl acetamide excepting four methyl hydrogen atoms. Axes are defined in Fig. 2*a* above.

Geometry optimization was not performed for the complex. The electronic charge distribution for the complex is shown in Fig. 2*b*.

DISCUSSION

Interaction of Na^+ with N-*methyl Acetamide*

From the results presented in Fig. 1, the minimum in energy for the Na^+ cation interacting with *N*-methyl acetamide occurs at 2.6 Å, a distance slightly greater than the sum of the van der Waals radius of the oxygen atom and the ionic radius of the Na^+ atom (20). The calculated minimum $Na^+ \cdots O$ distance of 2.6 Å differs by 0.2 Å from the $Na^+ \cdots O$ distance of 2.4 Å observed in the crystal structure of $NaBr \cdot 2CH_3CONH_2$ (21). However, the $Na^+ \cdots O$ distance of 2.6 Å is shorter by 0.1–0.2 Å than the limit established for sodium complexes with oxygen-containing organic compounds (22). The variation in energy with distance for Na^+ is less steep than for Mg^{+2}, where marked changes in the energy are observed around the minimum for even small departures from the optimal metal-ligand distance. The electronic charge distribution for the $Na^+ \cdots$ *N*-methyl acetamide complex at the optimal $Na^+ \cdots O$ distance of 2.6 Å is shown in Fig. 2*a* and reveals significant reduction in the charge on Na^+, from 1.0 electron units to 0.776 electron units in the complex. Such a reduction in the charge on the Na^+ cation has been recently considered with reference to a decrease in ion self-energy which would facilitate cation permeation through lipid membranes (16). There are significant perturbations of the electronic charges on the atoms in the ligand as a result of metal complexation.

Using the optimal $Na^+ \cdots O$ distance of 2.6 Å, *N*-methyl acetamide was rotated about the oxygen atom as described earlier. From the results presented in Fig. 3, an energy minimum was found to occur at $\theta = 50°$ on the nitrogen side whereas on the side of the carbon atoms the minimum was found to occur at 70°. The above minima correspond to Na—O—C angles of 130° and 110° for the cationic approach on the sides of nitrogen and carbon atoms of *N*-methyl acetamide, respectively. The above results are qualitatively similar to the results of *ab initio* calculations by Pullman (7), who found a slight preference for Na^+ on the C-methyl side of the ligand. Variation of the electronic charge on Na^+ with rotation of the ligand is found to be less steep than the change in electronic charge on Mg^{+2} with ligand rotation.

Interaction of Mg^{+2} with N-*methyl Acetamide*

The minimum in energy corresponds to 2.2 Å, approximately 0.05 Å longer than the sum of ionic radius of Mg^{+2} and van der Waals radius of the peptide oxygen (20). Unlike the interaction of Na^+ with *N*-methyl acetamide, the energy profile for Mg^{+2} interaction with the ligand shown in Fig. 1 is much steeper. The distance dependence of Mg^{+2} with a single *N*-methyl acetamide molecule was also investigated and the results of this study also showed the steepness of the energy-distance plot. Using the minimal distance of 2.2 Å, a clockwise and a counterclockwise rotation, of each of two coplanar *N*-methyl acetamide molecules related by a center of symmetry, were

performed. Rotations were about each Z axis, which is perpendicular to the ligand plane and which passes through each oxygen atom. The calculated results, presented in Fig. 3, indicate that the cation has a preference for an approach toward the C atom side of the carbonyl group.

The minimum in energy is found to occur at $\theta = 50°$ on the nitrogen atom side of the ligand and $\theta = 70°$ on the carbon atom side of the carbonyl corresponding to Mg—O—C angles of 130° and 110°, respectively. Such a result is in qualitative agreement with the conclusions of a recent survey of Ca^{+2}-carbonyl crystal structures made by Einsaphr and Bugg (23). The above authors found that the most common position of Ca^{+2} binding to a carbonyl ligand, as in a peptide moiety, is not along the C—O axis but rather in a torus with its center on the C=O axis. For Ca^{+2} complexes, the most commonly found position was on the side of the heteroatom with a C—O—Ca angle of ~140°. However, the calculations for Mg^{+2} interactions with *N*-methyl acetamide indicate the preferred position to be on the carbon atom side of the ligand.

There is recognized to be an overestimation of the binding energies using a very restricted basis set as in the CNDO/2 approximation. To estimate the reliability of the CNDO/2 binding energies for Na^+ and Mg^{+2} complexes with *N*-methyl acetamide, the interaction of both ions with one and two water molecules, respectively, was undertaken. For the interaction of Na^+ with a single water molecule, a minimum in energy was found to occur at 2.80 Å. The binding energy at the optimal $Na^+ \cdots O$ distance of 2.80 Å was 26.30 kcal/mol, close to the experimental value of 24 kcal/mol (24). For Mg^{+2} the binding energy was found to be 112.68 kcal/mol at a $Mg^{+2} \cdots O$ distance of 2.40 Å. The energies of the Na^+ and Mg^{+2} complexes were determined only at 0.2 Å intervals from a cation-ligand distance of 1.80–3.60 Å. The reliability of the binding energy for Mg^{+2} with two water molecules cannot be determined due to lack of experimental data. It is to be expected, as found, that the binding energies of both Na^+ and Mg^{+2} with *N*-methyl acetamide are much larger than the corresponding binding energies with a much smaller ligand like water. It is hoped with more extended basis sets (25) that the binding energies can be improved.

The calculation demonstrates that the CNDO/2 approximation yields reasonable $Na \cdots O$ and $Mg \cdots O$ distances for interaction with peptide carbonyl groups, although the sensitivity of the total energy to angular variation cannot be determined due to the paucity of experimental data. In this regard the CNDO/2 method can be used to obtain qualitative insight into the nature of interactions with biomolecules of second row alkali and alkaline earth metals, although caution must be exercised in correlating the total binding energies with experimental data (24).

It is to be emphasized that the CNDO/2 method provides at best a qualitative insight into cation-ligand interactions. Due to inadequate estimation of the van der Waals component (26) of the intermolecular interaction energy between the cation and ligand, the estimated binding energies are very likely overestimated. The simplistic model, i.e. CNDO/2, also precludes the estimation of contributions from correlation effects expected to cause a further reduction in the estimated binding energies. In-

clusion of the above terms in the intermolecular interaction energy will result in improved balancing of the different components, and it is anticipated will also improve the agreement between theoretical and experimental values for the binding energies.

Considerations of Ion Selectivity

When considering the binding of cations to peptide carbonyls in a biological milieu, one is usually concerned with competitive interactions with water. It can be said in general, however, that optimal binding to a peptide carbonyl yields more energy than optimal binding to a water molecule. Even so it should be appreciated that a water molecule, being small, can more readily approach the cation providing an optimal distance, whereas a peptide carbonyl, being part of a larger molecular construct, may be more limited in its approach and packing around the cation. Thus it is the capacity of a polypeptide or protein to present carbonyls at an optimal distance around a cation of a specific size that is limiting. When this can be achieved and when competition is between a divalent cation and a monovalent cation of the same size, the divalent cation will be preferentially bound (16).

Selectivity among the alkali or among the alkaline earth metal ions will depend on the ionic radius and on the capacity of the liganding system to present a first coordination shell of the appropriate size. It can be argued on steric grounds that, in general, it is more difficult for a polypeptide or protein to present the smaller coordination shell (16). When this is achieved, as for example in the highly selective and favorable binding of an Mg^{+2} or a Ca^{+2} ion, a complex structure is required (27).

Considerations of Lipid Membrane Permeation by Cations

Biomembrane permeation by cations is commonly discussed in terms of the Born ion self-energy expression $\Delta G\,(\text{Born}) = -Z^2e^2/2\epsilon r$, where ΔG is the Gibbs free energy, Z is the charge on the ion in electron units, e is the electronic charge of 4.8×10^{-10} esu, ϵ is the dielectric constant, and r is the radius of the ion. We have just seen by means of the CNDO/2 calculations that the charge on the ion is greatly reduced on complexation with peptide carbonyls. Therefore, even with the limitations of the above calculations, when considering ion permeation of a lipid bilayer by means of polypeptide carriers and channels, one cannot view the ionic charge of +1 or +2 as being uniformly localized within the ionic radius. The charge is nonuniformly delocalized over a radial distance that even includes the methyl hydrogens, as in the case of *N*-methyl acetamide (see Fig. 2), and the charge remaining on a divalent cation could be as law as about 0.5 electron units for six ligands. For a monovalent cation it is not unreasonable to expect that the charge is reduced to the level commonly found on atoms within the ligand itself.

From experimental transport data on the valinomycin carrier and the gramicidin channel it appears that the lipid dielectric barrier is not the dominating rate-limiting factor for a monovalent ion but the dielectric barrier may well be responsible for the lack of Ca^{+2} permeation through the gramicidin channel. A detailed analysis of this

aspect is given elsewhere (16). The charge reduction for a monovalent cation could well be sufficient to allow for multiple occupancy of a transmembrane channel.

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