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Potential Energy Fields about Nitrogen in Choline and Ethanolamine: Biological Function at Cellular Surfaces

Abstract. *Partial charge distribution on first and second neighbor atoms to nitrogen in choline and ethanolamine have been calculated. Coulombic and steric parameters were then utilized to evaluate the interaction of a negative test charge with the two molecules. Both the position and the magnitude of the maximum of interaction energy in the two systems were significantly different. The results suggest that ethanolamine interacts more strongly with anions than choline does. This is due principally to steric repulsion of the negative charge by the methyl groups in choline.*

Compounds containing choline and ethanolamine seem to influence characteristics of cellular surfaces. The distribution and amount of phosphatidyl choline and phosphatidyl ethanolamine are characteristic and specific in a particular membrane or cell (*1*). Furthermore, the charged functional groups of phospholipids appear to be located directly at the cell surface (*2*).

Material from the cell wall of pneumococcus contains a polysaccharide which carries convalently linked choline residues (*3*). Substitution of ethanolamine for choline in this cell wall significantly alters cellular adhesion, bacterial transformation, and cellular autolysis characteristics of this organism (*4*). Since at neutral *p*H the formal charge on ethanolamine and choline is the same, the difference in surface behavior might be due to differences in molecular size or conformation. However, the charge distribution could differ significantly in the two moieties and thereby provide a unique potential energy field about nitrogen in each molecule. We have investigated the latter possibility.

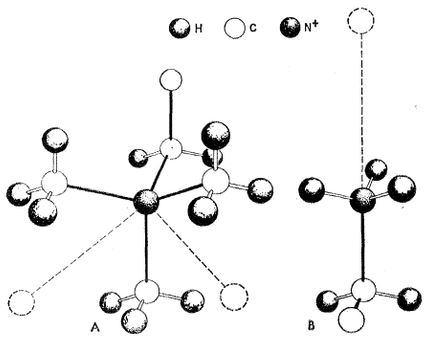

Fig. 1. Spatial location of maximum interaction (energy minimum) of a test anion with choline (A) and ethanolamine (B). Dotted circles represent the position of the negative test charge.

We evaluated the potential energy field about the positive nitrogen atom in the two molecules by using a test particle of negative charge and by calculating the total potential energy of the test particle at any position (x,y,z). This potential energy is approximated by the equation

$$P = \sum_{i=1}^{m} \frac{A_i}{r_i^6} + \frac{B_i}{r_i^{n_i}} + \frac{KQ_iQ}{D\,r_i} \qquad (1)$$

where

$$r_i = [(x - x_i)^2 + (y - y_i)^2 + (z - z_i)^2]^{1/2}$$

and Q_i is the partial charge on ith atom in the molecule; Q is the partial charge on test particle; D is the dielectric constant of the medium; A_i, B_i, n_i are the steric energy parameters of the ith atom interaction with the test particle; and m is the number of atoms in the molecule.

Partial charges on the atoms in the choline and ethanolamine molecules (Q_i) were calculated by the method of Del Re (*5*), which is based upon electron induction due to different electronegativities in different atoms. The partial charges calculated by this technique have been used to predict conformations of many polypeptides (*6–10*) and have also been used to predict nuclear magnetic resonance spectrums of amino acids (*5*).

The calculated partial charges on the first and second neighbor atoms to nitrogen in choline and ethanolamine are shown in Table 1. In choline, although there is some polarization due to the inductive effect, most of the positive charge is localized on the nitrogen atom buried within the methyl groups. In ethanolamine, most of the positive charge is distributed among the three hydrogen atoms attached to nitrogen. Thus, in ethanolamine the positive charge is more diffuse, but it is more accessible to approach by a counterion than that in the choline molecule.

With the values for Q_i shown in Table 1, we evaluated the potential energy field in each system according to Eq. 1. For this analysis, the molecules were positioned in the *trans*, all-staggered configuration (as in Fig. 1). The steric energy parameters were similar to the respective sets described by Scheraga, Flory, Liquori, and Huggins (*7, 8, 11, 12*). To include the effect of a solute-solvent medium on the final potential values, we assigned an effective dielectric of 3.5 to the coulombic potential term (*7, 8*). Although there is some criticism of the steric portion of the potential function (*12*), such "6-12" (or Lennard-Jones) functions have become widely accepted in the calculation of nonbonded interactions in gases (*13*), in the calculation of torsional potentials in various polymers (*14*), and in conformational analysis of polypeptides (*6–8, 11, 15*). However, the choice of an effective dielectric is uncertain and the value of 3.5 should be considered as only a crude approximation. Complete neglect of dielectric effects (vacuum) would only enhance energy differences, and the conclusions would not change.

A systematic digital scan was carried out by movement of the test negative charge in spherical coordinate space around nitrogen with the distance from nitrogen (r) varying from 2.5 to 6.0 Å at 0.1-Å intervals. Both spatial coordinates ϕ and θ were varied at 30° increments from 0° to 330°. The test charge

Table 1. Charge distribution in atoms around nitrogen in choline and ethanolamine.

Atom	Partial charge	
	Choline	Ethanolamine
Methylene carbon	−0.021	+0.003
Methylene hydrogen	+ .048	+ .054
Nitrogen	+ .685	+ .131
Methyl carbon	− .013	
Methyl hydrogen	+ .050	
Amine hydrogen		+ .248

Table 2. Radial position and energy of deepest energy minimums (maximum interaction) for varying test charge; r is the distance from nitrogen in angstroms; energies are expressed in kilocalories per mole.

Reaction to test charge									
−0.1		−0.3		−0.5		−0.7		−1.0	
Energy	r_{min}	Energy	r_{min}	Energy	r_{min}	Energy	r_{min}	Energy	r_{min}
				Choline					
−4.8	3.30	−10.3	3.20	−15.7	3.10	−21.2	3.00	−29.9	3.00
				Ethanolamine					
−4.0	3.00	−10.8	2.80	−17.8	2.80	−25.1	2.70	−36.1	2.60
				Difference					
+0.8	.30	−0.5	.40	−1.9	.30	−3.9	.30	−6.2	.40

was given steric (bulk) factors equivalent to those for the van der Waals radius of oxygen, and the negative charge was varied from −0.1 to −1.0.

The axes along which potential minimums occur are shown in Fig. 1. For the ethanolamine system, a single minimum occurs directly along the axis of the C–N bond. However, there are two minimums for the choline system, both on axes which bisect both tetrahedral angles at the nitrogen atom. Although there are three such axes, the third has a higher minimum because of the presence of the negative methylene carbon atom (second neighbor), which is *cis* to the test charge when it is brought in along this axis.

Table 2 shows the energies and the radial values for the minimums in the two molecules with different test charges. The energy minimums represent a combination of coulombic and steric forces, and the results reflect both parameters. The test charge reaches its energy minimum at radii which are consistently smaller for ethanolamine than for choline. This reflects steric repulsion by the choline methyl groups. Primarily because of this factor, the energy minimums are more negative for ethanolamine than for choline. However, as the coulombic part of the energy becomes less significant (smaller test charge), the energy differences become less and steric factors are most predominant.

These data do not take into account any polarization effects by atoms beyond those shown in Fig. 1. Polarization through such effects as internal salt formation are not considered, because definite information on the stereochemical aspects of cell surface components such as phospholipids is scarce. In regard to this point, Sundaralingham (*16*) has shown that crystalline α-L-phosphorylcholine glycerol and similar compounds are in a *gauche* conformation about the choline carbon-carbon bond. If this conformation exists in these compounds at cell surfaces, then the positions and magnitude of the energy minimums calculated here will be significantly altered. In any case, it is clear that both choline and ethanolamine possess distinct potential energy fields for interaction with anions, either through internal or external salt formation.

It is of interest to consider the probable effect of these differences on cellular adhesion. If adhesion depends on matching a surface charge with another surface charge, or on matching a surface charge with a linking macromolecule, then one would predict that membranes containing large amounts of ethanolamine relative to choline would adhere more strongly to one another. Negative sites apposed to a cell surface would be more strongly bound to ethanolamine-positive sites than to choline-positive sites. This is especially so if the negative sites carry a full or large negative charge on one atom, or can be polarized so that the negative charge is localized. Although the differences between the two systems become smaller as the negative charge is reduced (Table 2), these differences may well be significant down to values as small as 0.1 kcal/mole. Since surface charge matching is a cooperative effect, one must consider the sum of the interactions rather than the isolated case. An example of this type of situation is found in the work of Scheraga (*7*), who predicted the relative stabilization of right- and left-handed helices based on energy differences of less than 0.1 kcal per mole residue. The net energy difference clearly is the significant value.

In fact, Tomasz showed that cellular adhesion in pneumococcal cells with a high surface content of ethanolamine was far stronger than that in cells with a high surface choline content (*3*), an observation consistent with our results. Further alterations in surface properties may also prove to be due to the different potential energy fields about nitrogen in choline and ethanolamine residues.

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Human Growth Hormone Release: Relation to Slow-Wave Sleep and Sleep-Waking Cycles

Abstract. *Release of human growth hormone during sleep is significantly related to slow, synchronized stages of sleep and therefore would seem to be controlled by related neural mechanisms. When sleep-waking cycles are reversed by 12 hours, the release of growth hormone with sleep is reversed; thus release does not follow an inherent circadian rhythm independent of sleep.*

Human growth hormone (HGH) is spontaneously released during sleep without prior change in concentrations of glucose or insulin in plasma (*1, 2*) and in amounts comparable to maxi-