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Membrane Transport of Ammonia

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Abstract It is sometimes suggested that ammonium ions are deprotonated to form ammonia molecules so that the ammonia molecules can cross the non-polar membranes of cells. But given that ammonia in its non-ionised form is still quite polar it could be that ammonia shares membrane portals with ions or it could change into a non-polar configuration. In this experiment ammonia is examined with UV and NMR spectra to see if in non-polar environments, ammonia remains in a polar tetrahedral conformation or if it is transformed into a non-polar planar configuration so that it can easily cross non-polar membranes.

Keywords: *membrane transport, ammonia*

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1. Introduction

Ammonia production and calcium transport has been linked in studies looking at dietary protein induced hypercalciuria [1] and [2] and the connection between plasma insulin and calciuria [3] and the relation of ammoniagenesis and calcium transport [4]. It has been suggested that ammonia membrane transport could have an effect on calcium membrane transport. This study examines the mechanism of ammonia membrane transport and considers the possibility of it affecting calcium transport.

There is little reference in the literature as to membrane transport of ammonia. [5] Gannon (1977) describes ammonia transport as non-ionic diffusion. Non-ionic diffusion is used to explain diffusion of weak acids and bases across plasma membranes. In this explanation the ionised form of the material arrives at a membrane surface then it changes to its non-ionised configuration to allow diffusion through the nonpolar region of the phospholipid membrane. This description is satisfactory for weak acids and urea which have a non polar configuration in their molecular form. However, ammonia in its molecular form has a dipole moment quite similar to water i.e. molecular ammonia is polar: NH3 has dielectric constant of D = 1.46; H_20 has a dielectric constant of D = 1.84 compared with CCI_4, D = 0.0 [6] Zumdahl, Zumdahl (2013)Water is unable to diffuse across membranes by non-ionic diffusion and in fact requires protein bordered pores in membranes through which to pass. It seems impossible then for ammonia in its molecular form to diffuse as it is not nonpolar molecule. It may be that ammonia uses pores or transporters like water or it may be it alters its molecular configuration so as to produce an unknown nonpolar configuration. If the former is correct ammonia may compete with calcium for transport; but not so likely if the later idea is the true behaviour of NH_3.

Ammonia is very toxic due to the inability of cells to regulate its passage through membranes. If ammonia used pores or transporters, cells would have long ago evolved a means to regulate those apertures. But this has not happened so it may be that ammonia changes to a non polar form. This experiment describes a spectrophotomeric examination of ammonia in polar and non polar environments to look for any change in its molecular properties.

2. Method

Anhydrous ammonia gas was bubbled through dry carbon tetrachloride for 15 minutes while being shaken. An aliquot of the solution was then titrated with 0.01 N H_2S0_4 using methyl red indicators to determine the concentration of ammonia. For comparison of UV spectra an aqueous solution of ammonia diluted to that equal to the CCl_4 solution was used. For NMR spectra ammonia was bubbled through deuterated water in an NMR tube as well as through carbon tetrachloride.

UV spectra were measured using a Shimadsu dual beam recording spectrophotometer for both the aqueous and CCl_4 solutions of ammonia in each case water and CCl_4 solvents were used in the reference cuvettes respectively. [7] Jolly (1952).

For the NMR spectra the deuterated water solution and the CCl_4 solution when examined using a Varian EB360L NMR spectrophotometer and taking TMS as zero reference. [8] Weber and Thiele (1993).

3. Results

The NMR spectra showed that the absorbance of ammonia in D_2O was at 3.7 ppm and this was completely absent from the ammonia in CCl_4 solution. The CCl_4 solution of ammonia showed a small triplet of absorbance at 0.8, 0.25 and -0.5 ppm and a peak at 1.8 ppm that do not occur in the D2 O solution of ammonia (Refer Figure 1).

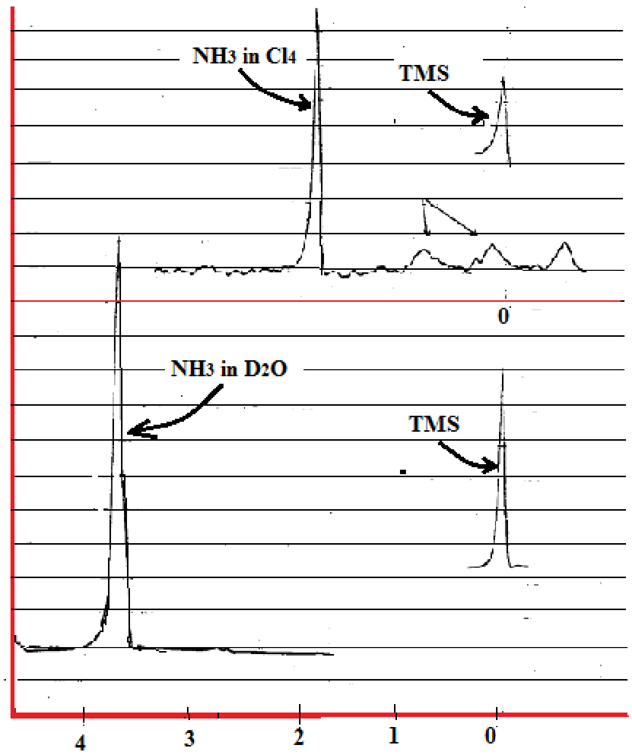

Figure 1. NMR of ammonia in D_2O and Cl_4

The UV spectra results show that such a conformational change occurs because the absorbance of light at 210 nm is removed in the Cl_4 solvent see Figure 2.

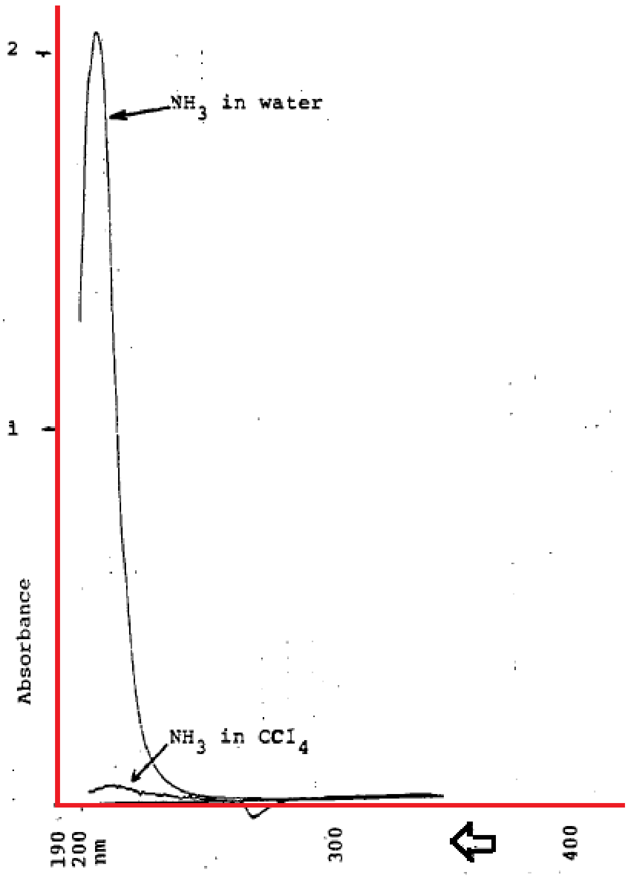

Figure 2. UV Spectra of ammonia in D_2O and Cl_4 solvents

4. Discussion

For ammonia to pass through cell membranes by non-ionic diffusion it must change to a nonpolar configuration. The normal molecular form of ammonia in liquid and crystalline ammonia has a tetrahedral configuration with the three hydrogen atoms extending down from the nitrogen to form a triangular pyramid [9] Morris and Boyd (1983) the apex of the pyramid is occupied by a lone pair of electrons. The negative charge on the lone pair of electrons and the positive charge in the three hydrogen nuclei are at opposite ends of the molecule and thus produce the dipole moment of D = 1.46. This configuration is explained by the electrons in the nitrogen being distributed into four equal tetrahedrally arranged Sp3 hybrid sp orbitals in the same manner as oxygen and carbon does in forming water and methane molecules respectively.

Spectroscopy of ammonia reveals that NH3 undergoes inversion i.e. turns inside out. There is only a small energy barrier of 2.5 kj slowing this inversion and ammonia can absorbed 210 nm light when it resonates between the two configurations.

In order to invert ammonia must pass through a higher energy intermediate were the lone pair of electrons are distributed equally on both sides of the molecule and the ammonia molecule is in a planar configuration with equal bond angles of 120°C. This intermediate configuration is most likely a transitory Sp2 hybridised form. Such a configuration is known to occur as the stable molecular form of BF3 with a dipole moment of D = O.

The Sp2 planar form of ammonia is least stable in polar solvents and liquid ammonia because of the interparticle forces of the surrounding particles that tend to stabilise the polar configuration. However in nonpolar solvents the stabilisation of the tetrahedral configuration is no longer available and the planar form is likely to be stabilised by hydrophobic bonding.

The results of this experiment confirm that such a conformational change occurs because the absorbance of light at 210 nm is removed in the nonpolar solvent. Indicating the molecule no longer inverts and the disappearance of the NMR absorbance at 3.6 ppm and the appearance of a peaks at 1.8 ppm and 0 ppm indicate that the H atoms have change their orientation.

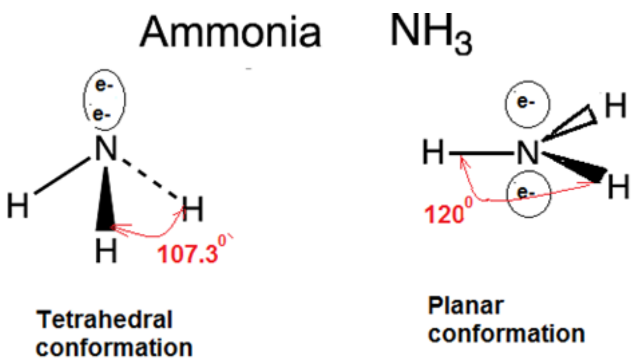

Figure 3.

Membrane diffusion of ammonia probably involves ammonia changing from the polar tetrahedral form at the cell membrane surface and assuming the planar non polar form for its passage through the nonpolar region of the membrane, reforming its polar configuration on the other side. Ammonia might be called an 'amphipolar' molecule because of its change from polar to nonpolar.

Because ammonia does not require transporters, pores or channels to cross membranes it is unlikely that it interferes with ions or polar molecules that do. It is therefore not likely that ammonia directly slows the passage of calcium into serial tubular epithelial cells.

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