

NMR evidence to change O/P ratio of water in presence of $-NH_2$ and $-NH_3^+$ spinomers

Vily Marius Cimpoiasu*

*Frontier Biology and Astrobiology Research Center,
University of Craiova, 13 A.I. Cuza Str., 200585 Craiova, Romania*

Abstract

We made liquid phase $^1H - NMR$ measurements of water solution of hydroxylamine (NH_2OH) and found deviation from natural ortho/para ratio of water due to presence of $-NH_2$ and $-NH_3^+$ spinomers. We propose that this difference can be used to study the chiral hydration of amino acids.

Keywords: *NMR*, amine, ortho/para.

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1 Introduction

Five types of $^1H^1HX$ nuclear spin isomers (spinomers) exist in aqueous solutions of primary amines: two N-amino isotopomers ($^{14}NH_2$ and $^{15}NH_2$) and three O-water isotopomers ($H_2^{16}O$; $H_2^{17}O$ and $H_2^{18}O$). Recent Time Domain $^1H - NMR$ results indicated that primary amine groups have higher affinity for $H_2^{17}O$ than for $H_2^{16}O$. It was proposed that this isotopic preference is due to a nuclear spin magnetic effect. Changes in $^1H - NMR$ signal amplitude (a proxy negatively correlated with the abundance of para-coupled protons) showed that the $^1H^1H$ -nuclear ortho:para ratio of $H_2^{17}O$ (OPR17) is significantly larger than OPR16. When OPR16 = 3.0 the OPR17 = 4.58 ([1]). Notably $-^{14}NH_2$ nuclear spinomers also have OPR values 3, ranging between 1.28 in ammonia and 3.445 in methylamine ([2, 3, 4]). Henceforth, the ortho and para states will not be evenly partitioned among the five different populations of $^1H^1HX$ nuclear spinomers present in aqueous solutions of primary amines (i.e. $^1H^1H^{16}O$; $^1H^1H^{17}O$; $^1H^1H^{18}O$; $^1H^1H^{14}N$; and $^1H^1H^{15}N$). Interactions between different nuclear spinomer pairs (i.e. ortho:ortho; ortho:para; and para:para) have different energy levels biasing the orientation of chemical groups and molecules as well as the equilibrium constants of chemical complexes ([5, 6, 7, 8]).

We propose that the $^1H^1H^{17}O$ -specific chelation seen during $^1H - NMR$ experiments on primary amines comes from the higher energy of interaction of the ortho-enriched $-^{14}NH_2$ for the ortho- $^1H^1H^{17}O$ (ortho- $^1H^1H^{17}O$). The large magnetic field applied in *NMR* is expected to amplify this magnetic interaction effect. The question posed here is whether $Bo = 0.5T$ (present on the surface of magnetite crystals) or $Bo = 1.5T$ (common in *NMR* measurements and easily produced with stable magnets) can create

*Email address: vilycimpoiasu@yahoo.com

sizable and measurable O-related isotopic anomalies in the population of water molecules from the hydration sphere of primary amines. Differences in nuclear spin:spin couplings between $R - NH_2$ and $R - NH_3^+$ groups are not easily to resolve; they depend largely on the nitrogen isotope, the nature of R and solution properties. We only discuss here the $-NH_2$ state of primary amines containing ^{14}N . All five isotopomers shown above are of $^1H^1HX$ -type (i.e. two protons close enough for spin:spin coupling and another nearby and equidistant nucleus, that may or may not intervene in the $^1H^1H$ spin coupling). In the simplest case the nuclear $^1H^1H$ spin coupling assumes three degenerate configurations called ortho with similar overall magnetic moment μ , and one para configuration with $\mu=0$. From the perspective of the model discussed here, only two states of each spinomer will exist: ortho and para.

To explain the basic principle of this analysis we start from a simple case of one solute (A) in two spinomeric forms ortho and para (A_o and A_p), hydrated with water containing a single O-isotopomer ($^1H^1H^{16}O$), also in two spinomeric forms (W_o and W_p). Ignoring presently the fact that each amine group has more than one water molecule in its hydration sphere, four types of XY spinomer:spinomer pairs may exist: A_oW_o ; A_oW_p ; A_pW_o and A_pW_p . The probability of formation of a specific spinomer: spinomer pair is proportional to:

$$p[A_iW_j] \sim \frac{(E_H)_{A_iW_j}}{kT} \cdot [A_i][W_j]. \quad (1)$$

where: $[A_iW_j]$ = the concentration of the A_iW_j spinomer:spinomer pair; i = ortho or para spinomers of the primary amine; j = ortho or para spinomers of water; $[A_i]$ = the concentration of the A_i spinomer; $[W_j]$ = the concentration of the W_j spinomer; $(E_H)_{A_iW_j}$ = the energy of interaction during the hydration of A_i with W_j . k = Boltzmann constant; T = temperature. Both the exchange kinetics and the equilibrium of spinomer:spinomer interactions are controlled by E_H/kT . Yet, if diffusion effects are ignored the ratio between the abundance of any two spinomer:spinomer pairs is kT -independent.

2 Experimental

Sample preparation

Our studies were carried out on hydroxylamine solutions. We made three solution of concentrated hydroxylamine: solution 1- 2.34M pH 10.6; solution 2- 2.52M pH 6.30 and solution 3- 2.47M pH 4.55. First we carefully dissolve hydroxylamine in Milli Q water and add precise amount of NaOH. After this stage, solution was stabilised 24h in thermostated chamber at 25⁰C. Before finish temperature stabilisation and after we measure carefully pH of solution. No deviation was recorded. We use pH for establish amount of $-NH_3^+$ and $-NH_2$ group in solution. All NMR sample was thermostated at 25⁰C for 2h in sample tube before NMR measurements.

NMR measurements

The time domain NMR measurements were performed on a 40 MHz low-resolution pulse 1H -NMR Spin Track spectrometer equipped with an audio filter bandwidth of 1 MHz and quadrature phase sensitive detector. NMR measurements were performed at constant temperature 25±0.2⁰C using the variable temperature unit. The standard TD-NMR (time domain) pulse sequences Carr-Purcell-Meiboom-Gill CPMG sequence [9, 10], were used to estimate the spin-spin relaxation time (T_2) values for protons in the sample. The repetition delay (RD) was set to 60 sec and the enhancement was 16 (256 scans). The total time for acquired of signal, corresponding to the various numbers of experi-

mental points, is experimentally establish by total relaxation of protons in sample. The duration of these CPMG curves allows a correct characterization of the slow relaxing components (water protons and exchangeable $-OH$ protons). We use various interpulse delay (t_0 -time interval between 90° pulse and 180° pulse in CPMG sequence; $100\mu s-4000\mu s$). Consequent on our previous work [11], regarding the stable reconstruction of T_2 distribution applied to NMR relaxation curve, we predict for all samples presented above, a multi-exponential distribution. The $T_{2,i}$, P_i values were calculated using this nonlinear regression program based on the Inverse Laplace transform algorithm, discussed earlier in [11]. For amplitude measurements we use an mineral oil etalon. This etalon was construct from hermetic glass bubble filled with mineral oil. Prior of amplitude measurements we calibrate the etalon with amounts Milli-Q water sample, and establish relaxation times of mineral oil an also we calculate etalon gain of NMR spectrometer: 109,881.81 units/ortho-mole of H. This glass bubble was measured before and after measurements of hydroxylamine solution. These two measurements was used to establish the gain of NMR detector. We calculate amplitude of each sample using the five exponential compartment model. At each pH we measure three probes; each probe was measured six times and we obtain an error less than 2% for each sample.

3 Results and Conclusions

We applied the following procedure: 1) Calculate the moles of H originate from the other ingredients, namely of water and the from $NaOH$. 2) Calculate the moles of H originate from each group of hidroxylamine, (OH , $-NH_2$ and NH_3^+). 3) We makes difference of amplitude as: experimental amplitude minus gain multiply with sum of moles of ortho- H from (water, $NaOH$, hydroxylamine OH , $-NH_2$ and $-NH_3^+$).

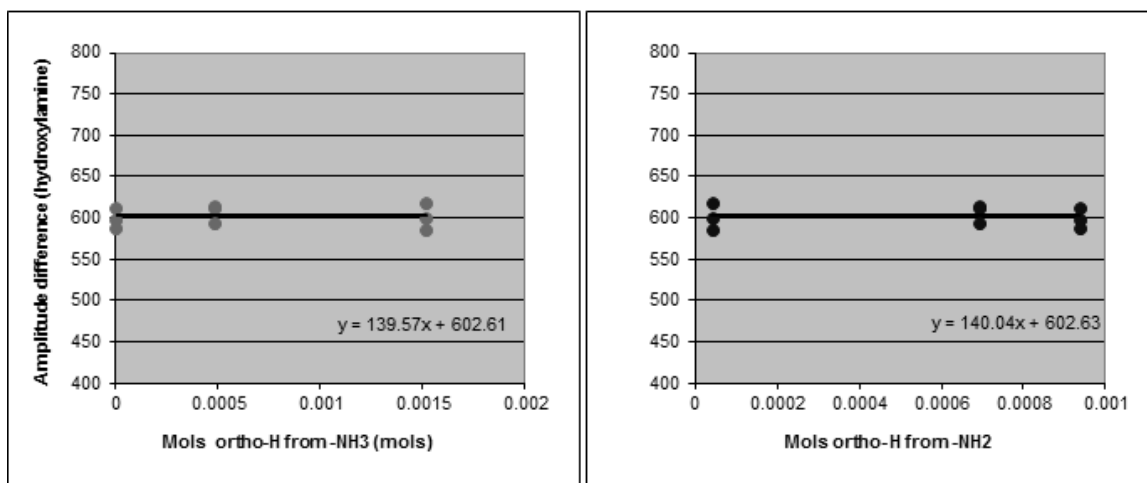


Figure 1: Amplitude difference versus moles ortho- H from amine groups

We notice that what remains is slow dependent on the moles of named ortho- H (from $-NH_2$ and $-NH_3^+$) (ie moles of H uncoupled or ortho in two radicals). The poor dependence also shows that importance of spinomer presence in water. At even small concentrations of spinomers, the effect appears .

In fact, in this circumstances, we do not know real OPR of $-NH_2$ and $-NH_3^+$. Whatever, we use a specific values of OPR dictated by positive and slow dependence from

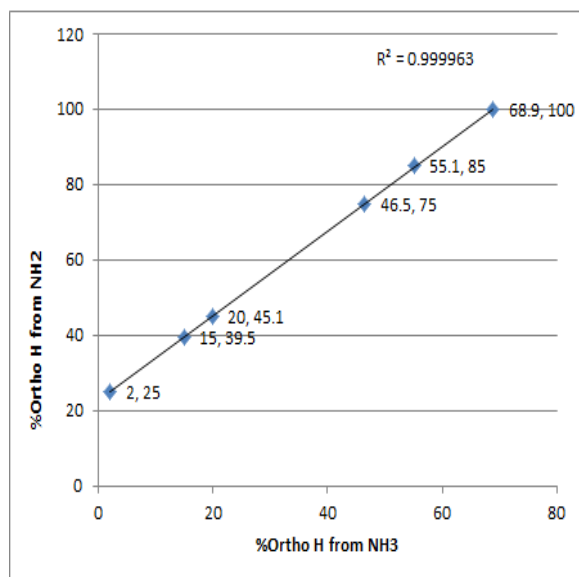


Figure 2: Linear dependence between populations of ortho- H from amine groups

Figure 1. The OPR values of $-NH_2$ and $-NH_3^+$ shows a linear dependence (see Figure 2).

In all cases we obtain positive difference (See Figure 1). This fact indicated that OPR of majoritar compartment of solution, water, was deeply affected by interaction with spinomers $-NH_2$ and $-NH_3^+$. in final, we calculate the deviation from natural OPR of water, and we obtain 36% more ortho-Hydrogens in water.

This model does not include ortho/para changes during hydration, complexity added by other hydrogen isotopes, magnetic interactions involving electron spins, field shield isotopic effects and ordering effects at interfaces (e.g. water surface and container margins). This observed asymmetry as dissimilar proton exchange during ^1H-NMR is not a direct consequence of pK dissimilarity between the water O-isotopomers [12]. Our results introduces a new mechanism that may be used to help explain differences in hydration between amino acid enantiomers seen earlier by isothermal titration calorimetry [13]. The presence of $-NH_2$ and $-NH_3^+$ spinomers in water, in presence of relative high magnetic field, induce a important variation in ortho/para ratio of water molecules.

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