FTIR analysis of ortho/para ratio in liquid water isotopomers. Implications for enantiodifferentiation in amino acids

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Abstract

We made liquid phase $FTIR$ measurements of water isotopomers and found dissimilar ortho/para ratio between $H_2^{17}O$ and $H_2^{16}O$. Results indicate lower abundance of para-coupled $^1H^1H$ spins in $H_2^{17}O$ relative to $H_2^{16}O$. We propose that this difference can be used to study the behavior of $H_2^{17}O$ during the chiral hydration of amino acids.

1 Introduction

Mechanisms and effects explored for explaining subtle differences between amino acid enantiomers include: ring currents [1][2][3]; neutral currents in weak interactions [3]; solubility [4][5]; magnetic properties of crystals [6]; crystallization of $Ni - AA$ complexes [7]; ortho/para ratio (OPR) [9]; chemical reactivity [8] and charge distribution in chiral centers in magnetic fields (B) [10]. Recent evidence indicated that dissimilar hydration complexes occur between enantiomers and water isotopomers, namely $H_2^{16}O$ and $H_2^{17}O$ [11][12][13]. This chirality may start from small differences in mirror symmetry regarding electro-magnetic organization between enantiocenters, but may also be controlled by differences in the abundance of different nuclear $^1H^1H$ spin pair types (e.g. ortho vs. para) between water isotopomers. The relative abundance of ortho and para in $H_2^{17}O$ should be affected by the ^{17}O nucleus (a AAX system of nuclear spins constructs with one large quadrupole (^{17}O), but this OPR partition of $H_2^{17}O$ is unknown and hard to predict. We analyzed differences in OPR between $H_2^{16}O$ and $H_2^{17}O$.

The nuclear $^1H^1H$ spin pair of water exists in ortho coupling (three states with total spin $I = 1$) or para coupling ($I = 0$). The abbreviation spinomers was proposed for spin-isomers [14]. Ortho and para nuclear spinomers confer molecules dissimilar physical-chemical properties [15]. At $\geq 50K$ OPR is ˜$3 : 1$ [16]. The kinetics of OPR equilibration is rapid [17]. Controllers of OPRs magnitude and of the ortho/para (OP) transition rate include temperature, electric and magnetic fields and neighboring nuclear spins [18][19]. Most commonly discussed mechanisms of OP transition are: spin conversion caused by

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magnetic interaction of ^1H spins with paramagnetic centers; spin conversion catalyzed by pressure; and spin conversion due to a H chemical exchange [20]. In free water molecules OP interconversion is forbidden or very slow, and if occurring is driven by weak nuclear spin/rotation interaction [21]. In $H_2^{16}O$ the energy difference between ortho and para is derived from the magnetic coupling constant averaged out by the spin exchange [22]. Thus, enrichment of pure $H_2^{16}O$ in ortho or para without coercing agents is seen by some as impossible [17][22]. In non-homogeneous B, nuclear spins are out of phase and this speeds up OP transitions. Single paramagnetic ions are sufficiently strong to change the OPR; [20][23]; notably, ^{17}O is paramagnetic and has a large nuclear quadrupole (0.26 barns)[24]. All above conditions affecting OPR involve alterations in the symmetry of electron shells, proton spin functions, spatial position of nuclei and rotational Hamiltonian. Thus, OP transitions are induced by a dipole-moment component through the deformation of the electron shell [25]. As the electron shell of the $H_2^{17}O$ molecule is deformed by the large quadrupolar asymmetric charge distribution of the $^{17}O'$s nucleus, this asymmetry favors changes in OPR and makes OP transitions faster. Through spin-spin interactions the spinomer state of $H_2^{17}O$ is expected to be different and more complex than $H_2^{16}O$.

Time Domain ^1HNMR ($TD-^1HNMR$) in asparagine and alanine revealed chiral-dissimilarity in proton exchange regarding parameters such as rate constant and intrinsic spin-spin relaxation time [12]. Because these differences were well correlated with changes in the concentration of $H_2^{17}O$ ($[H_2^{17}O]$) we proposed that this effect involves (among others) alterations of OPR in $H_2^{17}O$ [12]. In these paper we investigate the possibility to estimate OPR in water isotopomers.

2 Materials and Methods

^{17}O-rich water is not available commercially in high concentrations without increased $[H_2^{18}O]$ as well. To find which isotope (^{17}O or ^{18}O) is the most important in controlling the abundance of para we used water with different $\delta^{17}O/^{18}O$ isotopic signatures. $H_2^{16}O$ with $5.88M$ $H_2^{17}O$ and $0.555M$ $H_2^{18}O$ was obtained from Cambridge Isotopes (USA). The pH of all samples was 7 ± 0.1.All water samples were stored and read at room temperature.

3 Results

Gas $FTIR$ spectra for water isotopomers were obtained from HITRAN2008 (http:// www.cfa.Harvard.edu /HITRAN/HITRAN2008/) and liquid absorption measurements were made on $H_2^{16}O$ (~19 mM $H_2^{17}O$) and $H_2^{16}O$ with $5.88M$ $H_2^{17}O$ and $0.555M$ $H_2^{18}O$ in the $400-4000cm^{-1}$ using a Thermo Electron Nicolete $FTIR$ spectrometer, using 182 and 364 replicate readings.

Earlier analysis in water included calculating: OPR in $H_2^{16}O$, effects of ortho and para states on rotational-level energies of the water molecule, spectral predictions and direct spectral observations in IR and far IR [26][27][28][16][29]. Since exact calculations of absorption line amplitudes in the spectrum of water is a very complex problem, earlier workers preferred verifying whether measurements conform to trends shown by theoretical predictions, or were in agreement with other measurements [29]. The abundance of ortho and para, do however change the ratio between different IR absorption peaks, though this relationship is complex. We recorded and compared the IR spectra for $H_2^{16}O$ water with 19 mM $H_2^{17}O$ and $H_2^{16}O$ with $5.88M$ $H_2^{17}O$. Earlier IR works revealed the most

important diagnostic v_2 vibrational bands for discriminating ortho and para [26][29]. We analyzed the ro-vibrational bands from the $1660 - 1671 cm^{-1}$ domain.

Earlier work showed that changes in absorption of the different IR bands are influenced by changes in the abundance of ortho and para. We obtained IR peaks and their corresponding absorption intensities from $HITRAN08$ in the $0 - 25233 cm^{-1}$ range for $H_2^{16}O$ and in the $10 - 14437 cm^{-1}$ range for H217O. We identified transitions between energy levels, each representing a specific combination of quantum states J, K_a, K_c, v_1, v_2 and v_3. We identified bands corresponding to ortho and para using the rule $q = K_a + K_c + v_3$, which is even for para states and odd for ortho states [30]. OPR values were compared between $H_2^{16}O$ and $H_2^{17}O$ individually (i.e. one pair at a time) and collectively (i.e. all pairs within a wave number range).

For individual analysis we used pairs of ortho=>ortho and para=>para transitions, some reported earlier, some identified during the study(see below):

The transition pairs we have used are:

$3,0,3 \rightarrow 3,1,2$ (ortho) vs. $2,0,2 => 2,1,1$ (para) (identified in this study);

$3,1,2 \rightarrow 3,2,1$ (ortho) vs. $2,1,1 \rightarrow 2,2,0$ (para) (identified in this study);

$2,1,2 \rightarrow 3,0,3$ (ortho) vs. $1,1,1 \rightarrow 2,0,2$ (para) ([31]);

$7,1,7 \rightarrow 6,1,6$ (ortho) vs. $7,0,7 \rightarrow 6,0,6$ (para) ([32]);

$3,1,2 \rightarrow 3,0,3$ (ortho) vs. $1,1,1 \rightarrow 0,0,0$ ([16]).

For each pair we have analyzed all energy levels (i.e combinations of J, K_a, K_c and v_1, v_2, v_3. A total of 28 rations were calculated.

In the case of mid IR spectra obtained by direct observation we fit the experimental spectra with PeakFit 4.12 software. Because the ratio between these peak areas is not directly proportional with the OPR, but the direction of change is, we calculated the ratio between the o:peak area and p:peak area and compared $H_2^{16}O$ with $H_2^{17}O$. We predicted that we will find larger values for this ratio in ^{17}O-enriched water. Results confirmed that the ratio between the $1662.8 cm^{-1}$ o-band and the $1669.4 cm^{-1}$ p-band was 0.63 in $H_2^{16}O$ and 0.71 in 10.6 % $H_2^{17}O$ (see Figure 1 and Figure 2).

The collective analysis of all bands from $HITRAN08$ in the $2.26 - 24,991\ cm^{-1}$ range revealed only slightly larger OPR in $H_2^{16}O$ (OPR_{16}) (2.999058) relative to $H_2^{17}O$ (OPR_{17}) (3.001396). In the 010−000 band (corresponding with the range $7000 - 2,800 cm^{-1}$) OPR_{16} = 2.984686 and OPR_{17} = 2.999376. OPR differences between gas and condensed phases make it difficult to extrapolate from $HITRAN$ results to the liquid state $FTIR$ results. The resolution of liquid phase $FTIR$ is less than the resolution in gas phase $FTIR$, making some peaks hard to resolve and collective analysis of OPR almost impossible in liquid state. Tikhonov and Volkov (2002)[16] monitored changes in OPR_{16} in the $36 - 38 cm^{-1}$ range (gas phase), but did not measure OPR_{17} as well. We made direct $FTIR$ measurements ($400 - 4,000 cm^{-1}$) in liquid phase of $H_2^{16}O$ with 5.88M $H_2^{17}O$ and compared with $H_2^{16}O$. Most $H_2^{17}O$ peaks cannot be discriminated from $H_2^{16}O$ peaks. For gas/liquid $H_2^{17}O$ comparisons we used the transition $(010)432 - (000)321$ for ortho ($1788.027 cm^{-1}$) corresponding with $(010)331 - (000)220$ for para ($1766.728 cm^{-1}$). $HITRAN08$ gave OPR_{16} = 2.024 and OPR_{17} = 2.019, while the OPR_{17} in liquid phase was ˜2.7 (see Figure 2). The overall OPR_{17} in liquid phase could not be determined due to overlap of $H_2^{16}O$ and $H_2^{17}O$ peaks.

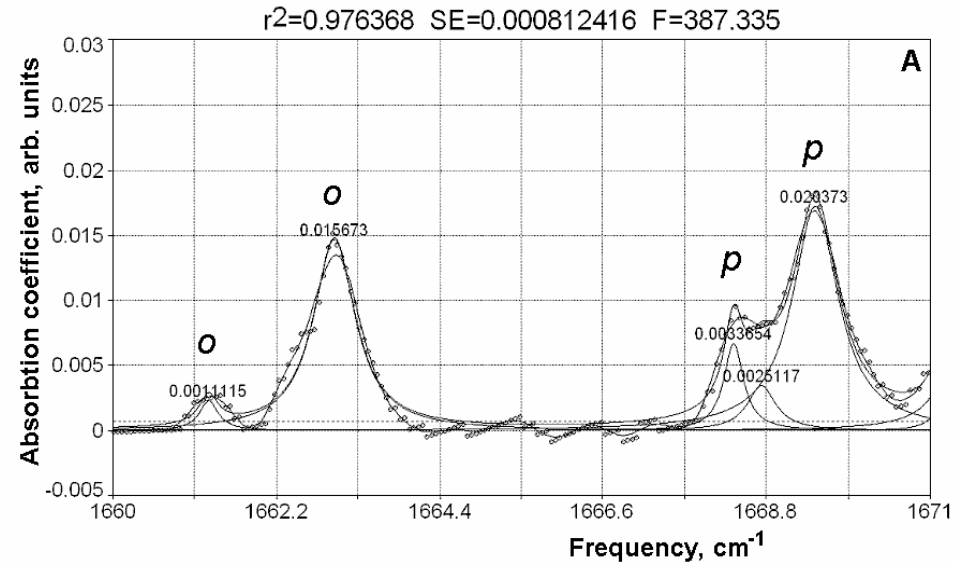

Figure 1: Spectrum fragment in the region of the v_2 vibrational band used in this work to compare the ratio between water ortho and para isomer concentrations for (A) $H_2^{16}O$. The area of peak is labelled on the top of peak. Strong absorption lines in the spectra of the ortho (o) and para (p) isomers are labeled by letters.

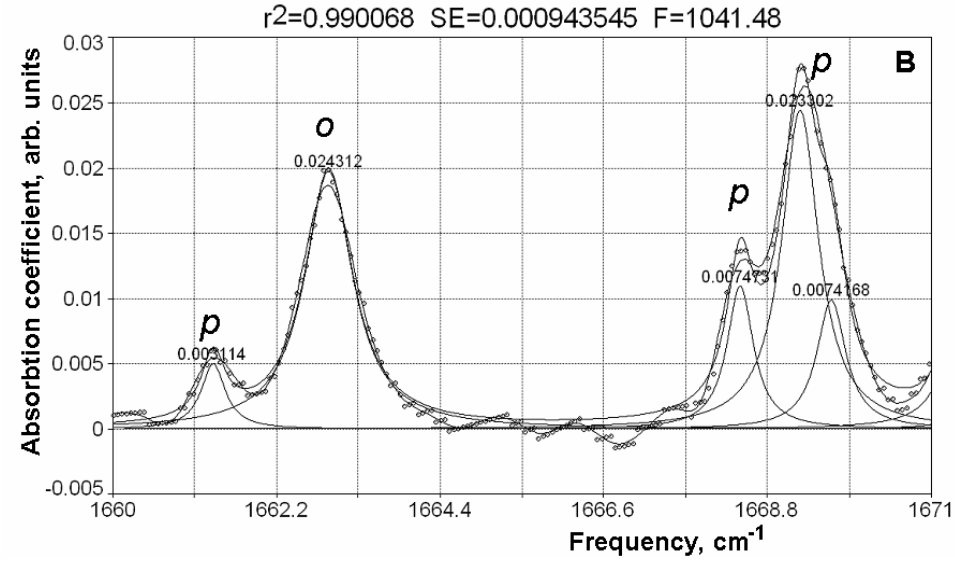

Figure 2: Spectrum fragment in the region of the v_2 vibrational band used in this work to compare the ratio between water ortho and para isomer concentrations for (B) 10% $H_2^{17}O$. The area of peak is labelled on the top of peak. Strong absorption lines in the spectra of the ortho (o) and para (p) isomers are labeled by letters.

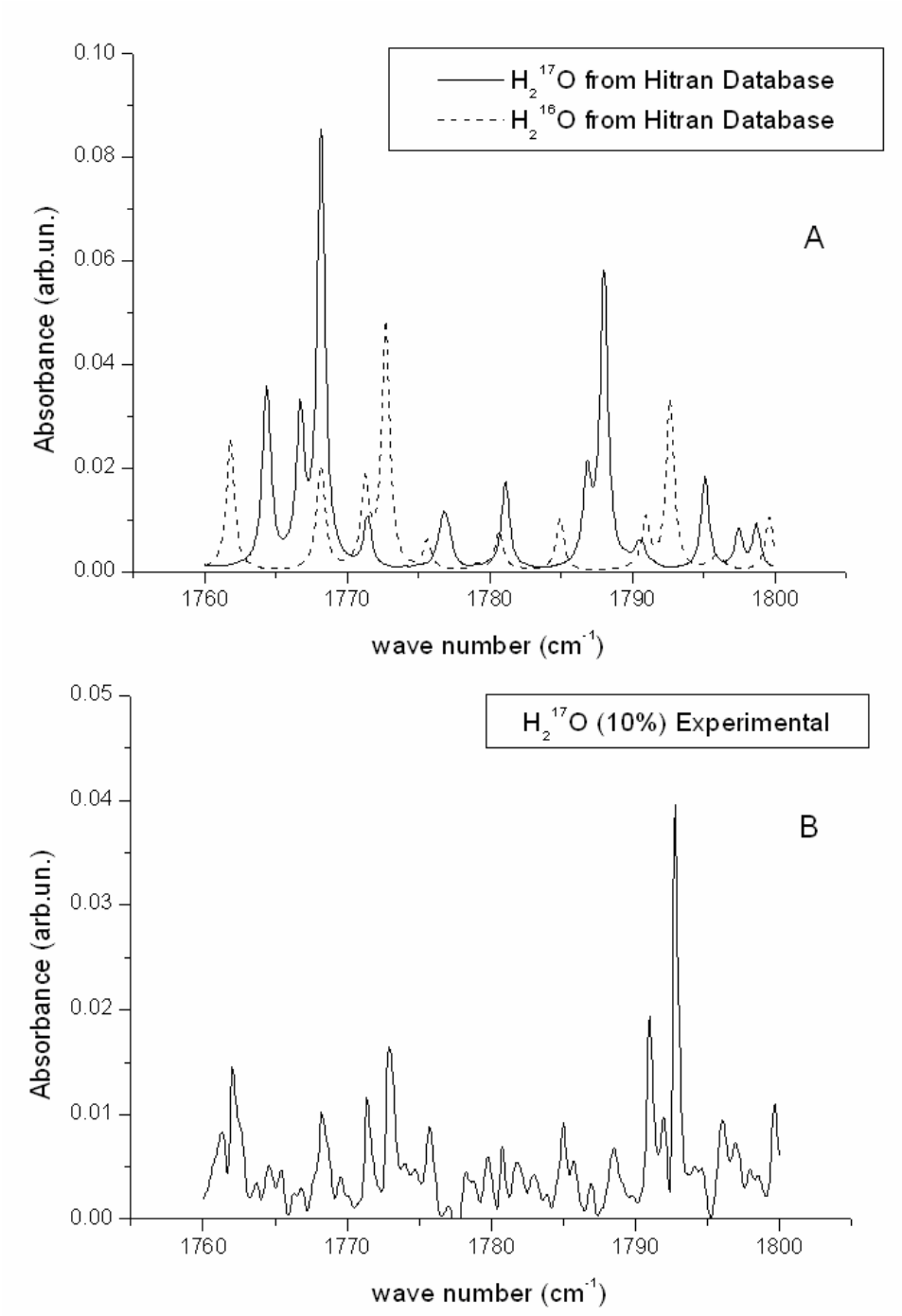

Figure 3: Spectrum fragment in the region of the v_2 vibrational band used in this work to compare the ratio between water ortho and para isomer concentrations for constructed spectrum from Hitran database for $H_2^{16}O$ and $H_2^{17}O$ (A) and FTIR experimental spectrum 10% $H_2^{17}O$ (B).

4 Discussions and conclusions

The simplest interpretation of our results is that there are less para and more "uncoupled" $^1H^1H$ nuclear spin pairs in $H_2^{17}O$ than in $H_2^{16}O$. In fact, the ^{17}O isotope leads to more complex $3s-AAX$ nuclear spinomers and thus the $O\&P$ states of $H_2^{17}O$ are not similar to the $O\&P$ states of $H_2^{16}O$. In water at $pH7$ the abundance of truly uncoupled spins (i.e. coming from OH^- and H_3O^+) is very low ($\tilde{}2\cdot 10^{-7}$) and not enough to influence the interpretation of these results.

We propose that the ortho:para differences between $H_2^{16}O$ and $H_2^{17}O$ are due to the specific properties of the ^{17}O nucleus, namely its electric quadrupole and magnetic moment. It was already shown that vicinity with paramagnetic compounds influences the OPR in H_2 [33]. Also, interaction with an external electrical field (with time dependent fluctuation) such as strog laser pulses leads to perturbations of the rotational states of water isomers [34].

These results have important implications for understanding interactions between nuclear spin isomers. Some chemical interactions are known to be $O:P$ discriminant. Para-water binds surfaces faster possibly because (unlike ortho) the para state can reach the zero-point rotational energy [35][25]. Also, the electromagnetic interaction between the magnetic moment of a pair of coupled spins and the magnetic moment of a chiral molecule are thought to have higher probability if spins are in ortho state [36]. If dissimilar OPR exists between enantiomers and between $H_2^{16}O$ and $H_2^{17}O$, then hydration complexes should be both isotopic- and chiral-dissimilar. This may explain L:D-OPR differences found earlier in amino acids [9][8]. Because of L:D dissimilar electro-magnetic organization and dissimilar para abundance in $H_2^{16}O$ vs. $H_2^{17}O$ hydration of enantiomers is mirror asymmetric. Thus, enantio-differences reported earlier during tyrosine crystallization may be attributed to L- vs. D-OPR asymmetry[4][5], but also to $H_2^{16}O$-$H_2^{17}O$-$^1H^1H$ pair coupling asymmetry. We did not discusse here the involvement of other water isotopomers such as D_2O and HDO, $H_2^{18}O$ and combinations of them. The mass spectrometry analysis bulletin of the ^{17}O-enriched waters we have used did not reveal increased concentration of D_2O or HDO.

Variations in the abundance of the nuclear spinomers ($H_2^{17}O$ and $H_2^{16}O$) have important implications for understanding the role of nuclear spin states of water in chemical and biochemical interactions.

The following interpretation of earlier findings is proposed. Dissimilar electro-magnetic organization exists between L- and D-enantiocenters [12]. It starts from the contraction of the $*C-H$ bond in the chiral center of amino acids generating an electron flow directed preferentially toward the nitrogen [37]. The Neutral Ring Current (NRC) thus established is not identical vis a vis electro-magnetic organization (i.e. direction relative to a given state of the molecule and relative to a moment in time). The $*C-H$ extension results in clockwise NRC in one enantiomer, while counterclockwise in the other [37]. When this effect is coupled with the magnetization of ^{17}O and $OPR_{16}\neq OPR_{17}$ chiral-asymmetric hydration of amino acids occurs with different water spinomers. This asymmetry is observed as dissimilar proton exchange during $TD-^1HNMR$ because the is a direct consequence of pK dissimilarity between the water O-isotopomers [38]; we expected this effect to apply for $H_2^{17}O$ as well. Our results introduces a new mechanism (namely $OPR_{16}\neq OPR_{17}$) that may be used to help explain differences in hydration between amino acid enantiomers seen earlier by isothermal titration calorimetry [39].

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