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Chemical Physics Letters 400 (2004) 117–121
CHEMICAL PHYSICS LETTERS
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Hydrogen bonding in complex of serine with histidine: computational and spectroscopic study of model compounds

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Received 16 August 2004; in final form 20 October 2004
Available online 11 November 2004

Abstract

We performed a comparative spectroscopic FTIR and computational study of the vibrational OH stretching frequencies in liquid ethanol–ethanol (**I**) and liquid ethanol–*N*-methylimidazole dimers (**II**). The latter system mimics the hydrogen bond formation between serine and histidine residues, which is the incipient step in the enzymatic activity of the catalytic triads. Complex (**I**) was studied as a reference system. The infrared spectra revealed the presence of the OH fundamental stretching transitions at 3339 and 3271 cm^{-1} for complexes (**I**) and (**II**), respectively. This red shift of 68 cm^{-1} indicates that the hydrogen bond between ethanol and *N*-methylimidazole exists and it is favoured over the one occurring in ethanol dimers. It is shown that vibrations exhibit anharmonicity.

1. Introduction

Hydrogen bonds are relatively weak interactions between molecules and yet they are of paramount importance in chemistry and pivotal in determining biomolecular structure and function. This type of interactions governs many biologically important processes in which the enzymatic catalysis is particularly relevant [1–3]. The incipient stage in the latter processes is formation of the hydrogen bonds between enzyme and substrate or hydrogen bonds between the enzyme residues. In typical biologically relevant reactions proton transfer is concerted with covalent bond rearrangements [4]. Enzymatic catalysis has been the focus of intensive research efforts, which have led to a penetrating insight into the mechanisms governing the reaction rate enhancement [1,5–9]. In a large number of enzymes the serine–histidine–aspartate (Ser–His–Asp) catalytic triad represents the active site [10]. The triggering step in catalysis involves creation of the hydrogen bond between serine and histidine, which depends on the basicity of the latter. The increased basicity of histidine in such triads was rationalized by low barrier hydrogen bond (LBHB) between histidine amino group and the carboxyl group of aspartate [7,10–12]. However, a closer scrutiny of the interactions between serine and histidine from both theoretical and experimental points of view is unfortunately lacking. In this Letter, we explore the nature of hydrogen bond between *N*-methylimidazole (NMI) and ethanol using vibrational spectroscopy and computational methods. In particular, we are interested in OH stretching frequencies of ethanol (EtOH) forming the hydrogen bond with other ethanol molecule versus the situation where the second ethanol is replaced by NMI as proton acceptor (Scheme 1). These two systems are

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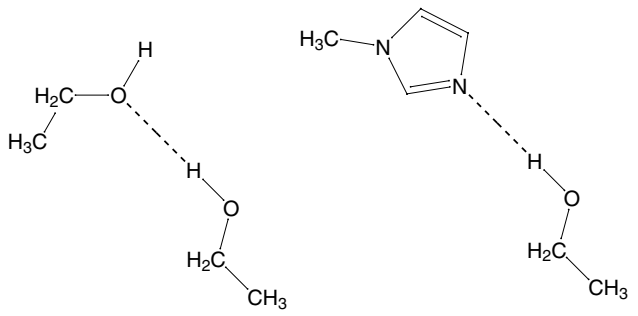
doi:10.1016/j.cplett.2004.10.089



I II

Scheme 1.

deliberately selected, because EtOH–NMI simulates the hydrogen bond formation among Ser and His residues, whereas EtOH–EtOH complex describes a Ser–Ser interaction and serves as a reference level. We believe that a study of the model compounds [13] will shed some light on the process taking place in the entire serine protease system, which in turn is too large and too demanding for both spectroscopic and computational treatment. The strength of H-bonding can be measured as stability of the complex vs. the separate parts as well as the change in the OH stretching vibrational frequency. The latter quantity is easily accessible from FT infrared spectroscopy.

The effect of deuteration on vibrational frequencies was investigated with per-deuterated ethanol. The experimental results are complemented by ab initio calculations. Vibrational frequencies of the OH and OD stretching modes were calculated in anharmonic fashion from the pointwise computed one-dimensional potential functions.

2. Experimental vibrational spectra

We performed measurements of the infrared spectra of bulk ethanol, 1:1 mixture of NMI with ethanol, bulk per-deuterated ethanol and 1:1 mixture of NMI with per-deuterated ethanol. An infrared spectrometer Perkin–Elmer System 2000 NIR FT-Raman was used. Chemicals were of analytical grade purchased from Merck (ethanol), Sigma Aldrich (NMI) and Cambridge Isotope Laboratories Inc. (per-deuterated ethanol). Preparative work was performed in a dry box in order to prevent traces of water in the samples. Spectra are shown in Fig. 1. OH stretching in bulk ethanol corresponds to the peak at 3339 cm^{-1}. Upon deuteration the peak is red-shifted to 2483 cm^{-1}. In the equimolar mixture of ethanol and NMI we assigned OH stretching peak to 3271 cm^{-1}, while there is still a peak corresponding to EtOH–EtOH complexes. The latter signal is weak indicating low population of such complexes.

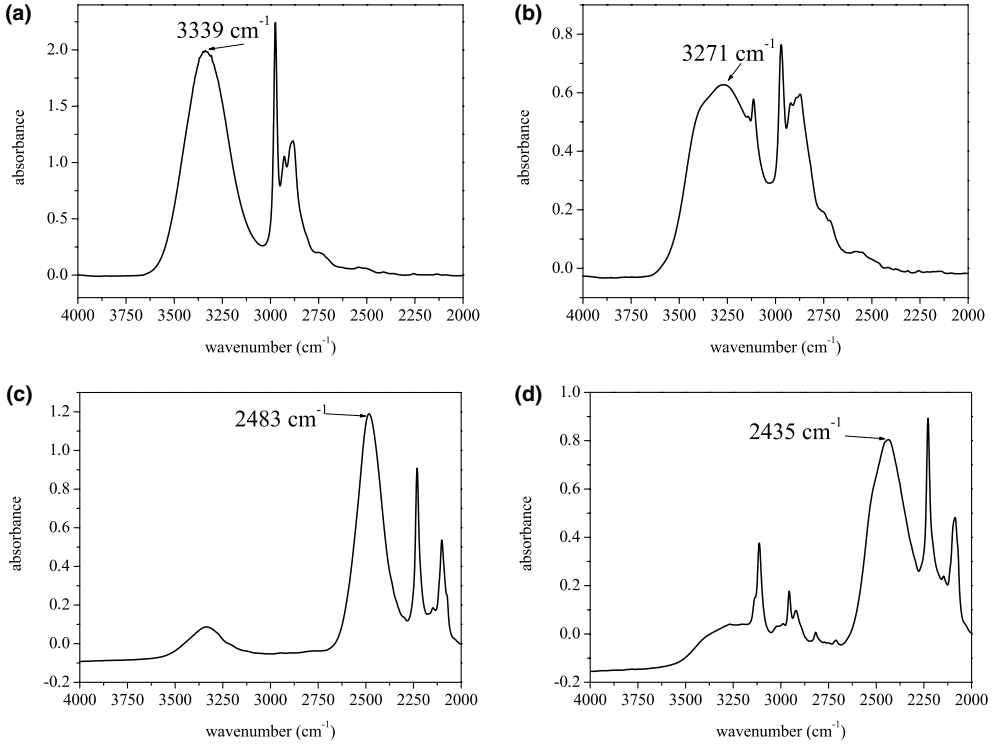


Fig. 1. Observed IR vibrational spectra of bulk ethanol (a), 1:1 mixture of ethanol and NMI (b), bulk per-deuterated ethanol (c) and 1:1 mixture of per-deuterated ethanol and NMI (d) in the region of 2000–4000 cm^{-1}. Assignment of characteristic OH stretching frequencies are shown on pictures.

The per-deuterated ethanol in the presence of NMI possesses OD stretching frequency at 2376 cm^{-1}.

3. Computational details

3.1. Quantum chemical calculation

It is well-known that computational studies of hydrogen-bonded systems require inclusion of the dynamical correlation effects and the use of the flexible basis sets containing diffuse and polarization functions [14]. For this purpose we utilized Møller–Plesset perturbation theory (MP2) and Pople's 6-31+G(d,p) basis set. Full geometry optimizations and frequency calculations within harmonic approximation were carried out at the MP2(full)/6-31+G(d,p) calculations (heretofore abbreviated as MP2) on both complexes (**I**) and (**II**). Interaction energies were calculated using the method of supermolecules and counterpoise method of Boys and Bernardi was applied to estimate the BSSE error. The employed level of theory is a compromise between the available CPU power and reliability of the results. Finally, we would like to mention that a complete treatment of the binding free energy of a complex in solution requires all atom simulation, which would be very demanding. A useful hint about the entropy contribution to the H bonding is provided by the gas phase calculations. Anticipating the forthcoming results, one can say that the calculated free energies reflect an increased H-bond strength in complex (**II**) compared to complex (**I**).

3.2. Solving vibrational Schrödinger Equation

The proton potential functions were obtained from the single-point energies MP2 calculations along the pathways extending from 0.703 and 0.708 Å to 1.453 and 1.458 Å away from the carboxylic oxygen in (**I**) and (**II**), respectively. The remaining geometric parameters of the complex in question were kept fixed. In both cases this involved 26 steps of the length of 0.03 Å. Cubic spline interpolation was applied to obtain the proton potential from the pointwise calculated energies. The OH stretching energy levels and accompanying wave functions in the one-dimensional potentials were acquired by solving numerically the one dimensional time-independent Schrödinger equation (SE) by the shooting method [15]. The details of the applied procedure were discussed elsewhere [15] being closely related to the well-established method introduced by Numerov [16]. The shooting program is written in standard FORTRAN 77 code and can be obtained from one of the authors (J.M.) upon request. All ab initio calculations were performed by using GAUSSIAN 03 suite of programs [17].

4. Results and discussion

Experimental and theoretical OH stretching frequencies are presented in Table 1. Infrared spectrum of bulk ethanol (**I**) reveals that the $0 \rightarrow 1$ transition of the OH stretching vibration can be assigned to 3339 cm^{-1} (Fig. 1). This fundamental band in spectrum is rather broad which is indicative of fluctuations of the polar environment coupled to the OH motion. Inclusion of liquid NMI with ethanol in a 1:1 ratio changes the appearance of the infrared spectrum. The most striking feature is the red-shifted frequency of the OH stretching in ethanol molecule towards lower frequencies (3271 cm^{-1}). The magnitude of the red shift is, therefore, 68 cm^{-1}. This observation would suggest that in the latter case a hydrogen bond between ethanol and NMI is formed and that it is favoured over the ethanol–ethanol hydrogen bonding. It follows that the OH force constant is slightly lower in (**II**) as compared with (**I**) implying that hydrogen bonding in (**II**) is somewhat stronger. These conclusions are corroborated by the gas-phase MP2 calculations on complexes (**I**) and (**II**). The optimized structures are depicted in Fig. 2. The hydrogen-bond strength is calculated as a difference in the total molecular energies of the dimer and its monomers. All binding energies were corrected for the basis set superposition error (BSSE) [18] using the counterpoise procedure [19] within GAUSSIAN 03 program. It appears that the hydrogen-bond strength, defined as a positive quantity, is 5.2 kcal/mol in (**I**), whereas it is increased to 7.0 kcal/mol in (**II**). The former value is in good accordance with the previously reported strength of 5.8 kcal/mol by Sandler et al. [20] obtained by MP2(fc)/aug-cc-pVDZ method. Inspection of geometric data reveals some additional interesting features. The OH-bond distance in the complex (**I**) is 0.973 Å, while the O···O separation is 2.860 Å. In the case of ethanol–NMI (**II**) complex the former bond is slightly elongated to the value of 0.978 Å, whereas the O···N distance is 2.891 Å. There are several criteria to assess the strength of hydrogen bonds. It is reflected in the distances between proton-donor and proton-acceptor atoms, OH stretching frequencies and chemical shift of the bridging hydrogen [21]. In cases of weak hydrogen

Table 1
Calculated and experimental OH stretching frequencies in investigated systems (in cm^{-1})

System	$\omega(OH)_{harm}$	$\omega(OH)^{SC}_{harm}$	$\omega(OH)_{anharm}$	$\omega(OH)_{exp}$
EtOH–EtOH	3732	3519	3443	3339
EtOH–NMI	3611	3404	3288	3271
EtOD–EtOD	2639	2488	2470	2483
EtOD–NMI	2553	2407	2376	2435

NMI denotes *N*-methylimidazole, while EtOH and EtOD stand for undeuterated and deuterated ethanol, respectively. Calculated values are obtained at MP2(full)/6–31+G(d,p) level of theory. The calculated scaled values are denoted by a superscript SC.

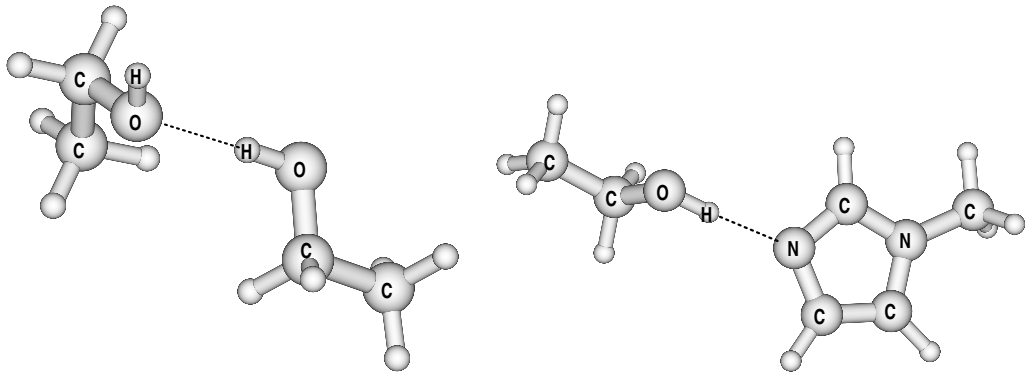


Fig. 2. MP2(full)/6-31+G(d,p) optimized geometries of complexes (**I**) and (**II**).

bonds, the covalent bond between H and O is typically 0.9–1.0 Å in length [22], and the heteroatoms are separated by more than the sum of their van der Waals radii, which for hydrogen bonded oxygen and nitrogen atoms implies distance $\geqslant$2.7 Å. Accordingly, the H bond between ethanol and NMI (**II**) falls in the category of weak bonds. This is evidenced by the O···N distance (2.891 Å), which is even larger than the sum of their van der Waals radii, the OH-bond distance of 0.978 Å and finally the calculated strength of 7.0 kcal/mol. A modest red shift upon the complex formation also indicates that the H bond is weak. The same holds for deuterated species. Deuteration of the parent ethanol changes vibrational infrared spectrum in both cases (Fig. 1). In the bulk per-deuterated solution, OD stretching frequency is found at the 2483 cm^{-1}. The observed isotope effect is therefore $\omega(OH)/\omega(OD) = 1.344$ implying that the potential for stretching is close to harmonic. In the 1:1 mixture of NMI an EtOD this vibrational mode is red-shifted by the extent of 48 cm^{-1} and pushed down to the value of 2435 cm^{-1}. The ability to adequately reproduce this numbers and to correctly predict the positions of the vibrational transitions in the IR spectrum is an important task for the computational chemistry. Hydrogen-bonded systems execute highly anharmonic vibrations and require calculations beyond the harmonic approximation. We performed vibrational analysis in the harmonic approximation in order to estimate the effects of anharmonicity. For this purpose the OH fundamental stretching frequencies are calculated by using several methods (Table 1) in both complexes. Firstly, we have calculated the MP2(full)/6-31+G(d,p) frequencies within harmonic approximation and obtained values of 3732 (2639) and 3611 (2553 cm^{-1}) for undeuterated (deuterated) complexes (**I**) and (**II**), respectively. This numbers are in sharp disagreement with the experimentally determined values. A somewhat better agreement could be obtained by applying the common frequency scaling factor of 0.9427 for this level of theory proposed by Scott and Radom [23] ($\omega(OH)^{SC}_{harm}$ in Table 1). However, the obtained frequencies are still not quite satisfactory. It is gratifying that the OX (X = H, D) stretching frequencies are much more precisely reproduced beyond the harmonic approximation using proton potential functions and by solving the time-independent vibrational Schrödinger equation applying the shooting method. The obtained values read 3443, (3288), 2470 and (2375) cm^{-1} for complexes (**I**) and (**II**) and their deuterated counterparts given within parentheses, respectively. Almost perfect agreement with experiment is found in the case of EtOD–EtOD and EtOH–NMI dimers, where the deviations from the experiment are 13 and 16 cm^{-1}, respectively. Considerable improvement by 76 cm^{-1} is attained in the EtOH–EtOH complex. Surprisingly, the calculated anharmonicity correction worsens agreement with experiment in EtOD–NMI complex for reasons unknown at present. It is fair to say, however, that the calculated anharmonic data are in much better accordance with experiment as a rule, which lends credence to the applied approach. The graphical representation of the proton potentials in the system EtOH–NMI and its vibrational eigenfunctions and vibrational eigenvalues are presented in Fig. 3. It appears that the proton vibrates in a single-well potential.

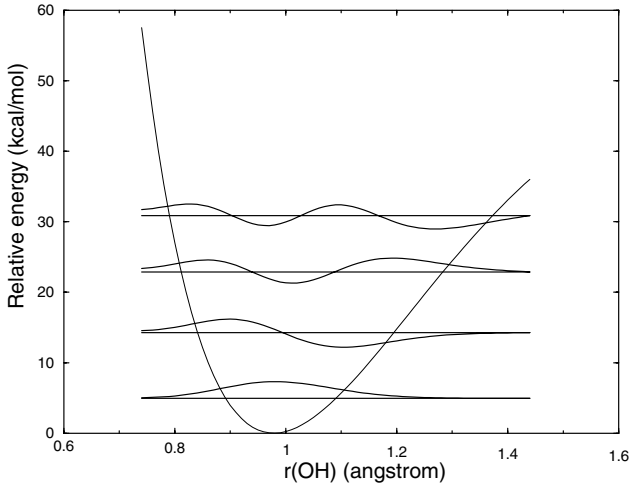


Fig. 3. Proton potential in ethanol–*N*-methylimidazole (**II**) complex, together with the first four OH vibrational stretching levels as well as corresponding wavefunctions. Proton potentials were calculated at MP2(full)/6-31+G(d,p) level.

5. Conclusion

The hydrogen-bond formation between the model compounds NMI and ethanol, which are selected to mimic the histidine–serine complex, is explored by the FTIR technique and ab initio MP2 method. It was demonstrated that hydrogen bonds between EtOH and NMI (**II**) are favoured over the ethanol dimers (**I**), meaning that the former are stronger. Both of these complexes, however, exhibit weak hydrogen bonding, as evidenced by hydrogen-bond strengths of 5.2 and 7.0 kcal/mol, theoretically estimated for (**I**) and (**II**), respectively, at the MP2(full)/6-31+G(d,p) level of theory. The evidence for formation of hydrogen-bonded dimer of EtOH and NMI is provided by the IR spectrum, where the OH stretching frequency is red-shifted by 68 cm^{-1} relative to the ethanol dimer. The isotope effects in the observed frequencies were obtained by repeating the experiment with the per-deuterated ethanol. The relative values of such vibrational OD frequencies are lower, as intuitively expected. The red-shift was also observed in this case, but to a smaller degree (48 cm^{-1}) in deuterated EtOD–NMI system. In order to obtain a good accordance with the experimental frequencies, vibrational analysis beyond the harmonic approximation was necessary. The estimated anharmonic corrections improved the quality of the calculated frequencies for H bonds as expected.

The present results are complementary to recent spectroscopic work of Frey and co-workers [13], where Asp–His part of the triad was considered. Our combined FTIR and ab initio study shows that the hydrogen bond between His and Ser should be weak, but stronger than in the Ser–Ser system. Another important finding is that the vibrational potential for the proton is described by a single well. It should be strongly pointed out that specific interactions in the real serine proteases could lead to substantial changes in the local pK_a values leading to modified H-bond strength, changes in vibrational frequency and a different shape of the proton potential. These interactions can be in principle recast into the dielectric constant at the active site. The value of the dielectric constant in that region is still a subject of debates [24,25]. However, it is safe to say that in the real enzyme the proton potential is changed in a way that proton transfer is thermodynamically feasible and takes place together with the rearrangements of covalent bonds. A comprehensive description of this process requires a lot of efforts and application of the computational methods which include full fluctuating enzyme environment together with solvent and application of mixed quantum–classical molecular dynamics [5,7,26–31]. Unlike in proteins [32] and rigid systems [33], the NMR technique is not suitable for studying the EtOH–EtOH and EtOH–NMI complexes due to a fast exchange of molecules.

Acknowledgements

We thank Ministry of Education, Science and Sport of Republic of Slovenia and Croatian Ministry of Science, Education and Sport for financial support in the framework of the bilateral scientific cooperation agreement. We are grateful to Ms. Silva Zagorc for her assistance in experimental work and Dr. Jože Grdadolnik, National Institute of Chemistry for many stimulating discussions and critical reading of the manuscript.

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