# Study of hydration of sarcosine, formation of its zwitterion and their different oligomers in aqueous media: A Raman spectroscopic and theoretical study

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HIGHLIGHTS

- Combined Raman and DFT techniques applied to hydrogen bonds.
- Hydrogen bonded complexes optimized with many water molecules.
- The effect of hydration was studied.
- Concentration dependent peak shift and linewidth changes revealed hydrogen bonds.

GRAPHICAL ABSTRACT
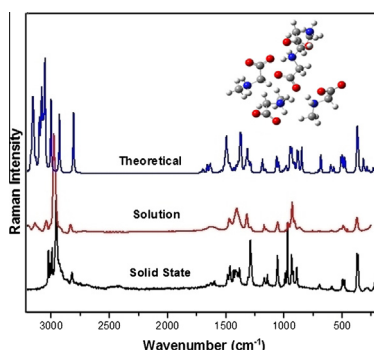
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ABSTRACT

Raman spectra of the biologically important molecule sarcosine (SAR) ($C_3H_7NO_2$) were studied experimentally in aqueous solution at different concentrations. These spectra were also calculated theoretically using density functional theory (DFT) at the B3LYP/6-311++G(d,p) level. Further, all the observed normal modes were assigned through potential energy distribution (PED). Geometry optimization of SAR produced its three conformers with slightly different energies. The lowest energy conformer of SAR was selected for a systematic solvation study wherein different numbers of water molecules (nW, $n = 1–9$) were placed near it. In the SAR-9W complex, the SAR molecule is located almost at the center of the cage of 9 water molecules. Geometries of different oligomers of SAR (dimer, trimer, tetramer and pentamer) were also optimized in aqueous media taking the input structures from crystallographic data and using the polarizable continuum (PCM). Proton transfer required for the formation of the zwitterionic form of SAR was found to occur when the number of water molecules in the first hydration shell was six or more.

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Introduction

Sarcosine (SAR) ($C_3H_7NO_2$) is an N-methyl derivative of the simplest amino acid, glycine. It is an amide and is found in muscles and other human body tissues [1]. It has also been found in star fish and sea urchins. Sarcosine (SAR) has been used as a biomarker for prostate cancer progression [2,3]. The role of SAR in improvement of memory in schizophrenia patients has been investigated [4,5]. In solution in a polar solvent as well as in crystalline phase, amino acids usually exist in zwitterionic form [6,7]. The crystal structure of SAR was determined by X-ray diffraction and it was found to exist in the zwitterionic form in solid phase. [8]. Hydrogen bonding is an important stabilizing force both in aqueous as well as crystal phases. In crystal phase, packing forces play important role

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producing significant effects on the geometries of the molecules and their complexes. The solvation of amino acids in aqueous media takes place due to proton transfer from the α-carboxylic group to the α-amino group, which results in the formation of their zwitterions [9–11]. A hydration study gives information regarding relative stabilities of the various conformers of a molecule in water which can be used to investigate its structure–function relationship. Several theoretical studies have been carried out on interaction between amino acids and water molecules [12–15].

From a study of near edge X-ray absorption fine structure spectroscopy of aqueous solvation of SAR, Uejio et al. [16] reported a blue shift in the SAR-N atom k edge spectrum which results due to methyl group substitution on the *N*-atom of SAR. Subsequently, theoretical and experimental studies were carried out on conformations of SAR and its hydrogen bonding interactions were studied using ab initio and DFT methods combined with FT-IR spectroscopy [17]. Later, in matrix isolation infrared and theoretical study employing DFT and MP2 methods, Gomez-Zavaglia and Fausto [18] reported the most stable conformer of SAR along with its vibrational analysis. Chaudhari et al. [19] studied hydrogen bonding interaction of SAR with one water molecule using three different methods i.e. Hartree Fock, MP2 and DFT and they also investigated the effects of different basis sets. Recently, a vacuum UV photon induced ionization and fragmentation of SAR was studied by Guo et. al. [20] and they reported the possible fragmentation pathways of different conformers of SAR. The G3B3 and B3LYP/6-311++G(d,p) methods were employed for calculating the reaction pathways to interpret the experimental results. In a most recent FT-IR work on SAR [21], the hydration effect on methyl derivatives of glycine has been reported. In this work Raman spectra were recorded and analysed to characterize the zwitterion and interaction of water molecules with the carboxylic and amino groups of SAR.

In view of the above mentioned studies, pharmaceutical and biological importance of SAR, it was considered desirable to study those aspects of it both theoretically and experimentally which have not received adequate attention. In the previous work [19], only one water molecule was considered to be associated with SAR through hydrogen bonding. Water is involved in interaction with various types of biomolecules e.g. amino acids and it plays an important role in living systems. Solvation of a solute molecule in aqueous media can be studied by permitting several water molecules to interact with it. One can also study this aspect by using a model for solvation in the bulk solvent, e.g. the polarisable continuum model. Zwitterions of an amino acid formed in aqueous media can also form higher aggregates e.g. dimer, trimer etc. To the best of our knowledge, these various aspects of solvent effect of aqueous media on Raman spectra of SAR and the corresponding aspects in solid state have not been studied yet. In this work, these various aspects including concentration dependence of the Raman spectra of SAR in aqueous media were investigated.

Methods

Theoretical methods

Geometry optimization and vibrational frequency calculations on SAR and its hydrogen bonded complexes with water molecules was performed in gas phase using the Becke's [22] nonlocal three parameter exchange and correlation functional along with the Lee–Yang–Parr correlation functional (B3LYP) [23] and the 6-311++G(d,p) basis set. In some recent studies [24,25] in our group, DFT calculations at this level were found to yield useful results which proved to be very crucial in interpreting several experimental observations. The calculated total energies were corrected for the basis set superposition error (BSSE) using the counterpoise

(CP) method of Boys and Bernardi [26]. To incorporate bulk solvent effect on the properties of SAR, the polarisable continuum model (PCM) [27] was employed. In PCM [27], the solute was placed in a cavity formed in the continuous solvent medium and both the solute and solvent polarize each other producing a charge distribution at the cavity surface which perturbs the solute. Vibrational frequencies of SAR, its hydrogen bonded dimer, trimer, tetramer and pentamer as well as its hydrogen bonded complexes with varying numbers of water molecules were studied in the harmonic approximation. All the theoretical calculations were carried out using the Gaussian 03 program [28]. For visualization of optimized structures and vibrational modes, the GaussView program was employed [29].

Experimental details

SAR purchased from Sigma–Aldrich with 99.9% purity was used without further purification. Raman spectra of SAR in powder form and at different concentrations in water were recorded in the region 250–3200 cm^{-1} using a SPEX 1404 double monochromator with 2400 grooves/mm grating. The signal was detected by a liquid – N_2 cooled CCD camera (Photometrices model) with scanning multichannel detection scheme. The 514.5 nm line of argon ion laser was used for excitation of Raman spectra with the operating power of $\sim$200 mW. The grating positions were kept constant to eliminate reproducibility error. The entrance slit width was kept at 50 μm which gave the spectral resolution $\sim$0.3 cm^{-1}. The integration time for spectral collection was 20 s per acquisition and number of scan was 2. The scattered signal was collected at 90° scattering geometry. The software used for collecting the spectra and analysis was Map2 from photometrices model.

Results and discussion

Zwitterion of SAR, SAR-nW (n = 1–9) complexes and different oligomers

To examine the effect of aqueous media, 1–9 water molecules were placed in a systematic way near SAR so that these can interact with it through hydrogen bonds. The geometries of these hydrogen bonded complexes and those of its different oligomers (dimer, trimer, tetramer and pentamer) were optimized at the B3LYP/6-311++G(d,p) level of theory. Interaction of water molecules with SAR stabilizes its zwitterionic form preferentially over the canonical form as found for other amino acids also [10,11]. Conformational analysis of SAR has been performed by other authors [18]. Three stable conformers of SAR were obtained by rotation about C—C, C—N and C—O bonds. The lowest total energy conformer of SAR (Fig. 1a) (total energy = −323.84125 Hartree) was used for constructing initial structures of SAR-water complexes. The zwitterionic form of SAR (Fig. 2c) was also optimized in aqueous medium employing PCM at the B3LYP/6-311++G(d,p) level of theory. The zwitterionic structure of SAR was found to be more stable than the canonical form in aqueous media by about 16.5 kcal/mol. The dipole moment of the zwitterionic form of SAR (14.7 D) was found to be much larger than that of the canonical form (2.3 D) in gas phase. A large dipole moment of zwitterionic SAR would play an important role in stabilizing it in the aqueous medium. In addition to the B3LYP/6-311++G(d,p) study, we also optimized the structures of both the canonical and zwitterionic forms of SAR in aqueous medium using MP2 and HF methods along with the 6-311++G(d,p) basis set. The bond lengths and bond angles of zwitterionic form of SAR obtained using HF, MP2 and DFT (B3LYP) methods are compared with the experimental XRD data [8] in Table 1. RMS errors between experimental and theoretical values