

A theoretical and matrix-isolation FT-IR investigation of the conformational landscape of N-acetylcysteine

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ABSTRACT

The conformational landscape of N-acetylcysteine (NAC) has been investigated by a combined experimental matrix-isolation FT-IR and theoretical methodology. This combination is a powerful tool to study the conformational behavior of relatively small molecules. Geometry optimizations at the HF/3-21 level resulted in 438 different geometries with an energy difference smaller than 22 kJ mol^{-1} . Among these, six conformations were detected with a relative energy difference smaller than 10 kJ mol^{-1} at the DFT(B3LYP)/6-31++G** level of theory. These were finally subjected to MP2/6-31++G** optimizations which resulted in five minima. The vibrational and thermodynamical properties of these conformations were calculated at both the DFT and MP2 methodologies. Experimentally NAC was isolated in an argon matrix at 16 K after being sublimated at 323 K. The most stable MP2 form appeared to be dominant in the experimental spectra but the presence of three other conformations with $\Delta E_{\text{MP2}} < 10 \text{ kJ mol}^{-1}$ was also demonstrated. The experimentally observed abundance of the H-bond containing conformations appeared to be in good accordance with the predicted MP2 value.

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1. Introduction

Structure determination of proteins is a challenging research field, because their conformation is of huge importance to understand key biological processes. Since amino acids are involved in peptide bonds which affect protein conformation, they are among the most important biomolecules. Proteins are too large to be subjected to high-level computational studies, but amino acids may be studied as model molecules. As a matter of fact, the amino acid side-chain influences the protein conformation and consequently also the thermodynamic and vibrational characteristics. Amino acids have been subjected to many theoretical and spectroscopic studies in their neutral and zwitterionic form. All α -amino acids occur in the zwitterionic form in the solid state and in solution, and therefore also in biological environment, whereas they exist in their neutral form in the gas phase. In proteins amino acids occur as a neutral residue of their ι - α -form.

For a better understanding of the conformational behavior of proteins and amino acids involved in the peptide bond, we are currently investigating N-acetylated amino acids. Due to the amide bond, these molecules serve as model system for the C-terminus amino acid in peptides. Matrix-isolation infrared spectroscopy is a suitable technique to study amino acid residues, since isolated spectra simulating the gas phase allow to directly compare exper-

imental results to ab initio spectral data. On the other hand, the latter calculations are necessary for a reliable interpretation of the spectra, which are rather complicated due to the coexistence of different conformations.

N-acetyl-L-cysteine (NAC) is the N-acetylated derivative of cysteine, the only natural occurring amino acid with a reactive sulfhydryl group [1]. The SH group is easily involved in S–S bridges which stabilize proteins from their primary to the quaternary structure [2]. NAC is a commercially available drug, mainly used as mucolytic agent and in the management of paracetamol overdose [3]. Recent investigations on the molecule have revealed that it reduces the cravings associated with chronic cocaine use [4,5], the symptoms of schizophrenia [6] and bipolar disorder [7]. It has also been demonstrated that an overdose of NAC, which is found in many body building supplements, potentially damages heart and lungs [8].

Similar to other amino acids, NAC possesses a significant number of internal rotation axes (Chart 1) which results in a large conformational flexibility. This implies that several low-energy conformations may exist in the gas phase. Pecul has theoretically investigated the neutral and zwitterionic structures of cysteine stabilized by four water molecules [9] and has simulated the vibrational Raman optical activity (ROA) spectrum [10]. The conformations of cysteine have been theoretically predicted at the density functional theory (DFT), including Becke's nonlocal hybrid three-parameter exchange functional (B3), in combination with the Lee–Yang–Parr (LYP) correlation functional level by Dobrowolski

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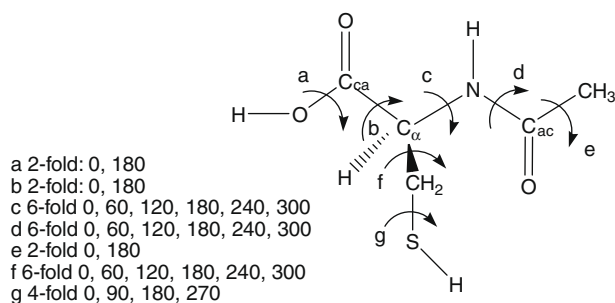


Chart 1. Possible internal rotations (deg) in N-acetyl-L-cysteine.

et al. and some of these have been experimentally observed in N_2 and Ar matrices [11]. The same authors have also examined the cysteine conformational behavior with the Møller–Plesset second-order perturbation (MP2) method where 51 stable conformations have been found in the gas phase and the influence of water has been estimated in a study by IEF-PCM (the integral equation formalism version of the polarizable continuum model)/B3LYP/aug-cc-pVDZ calculations where 44 neutral and 12 zwitterionic stable cysteine conformations have been found [12]. In contrast to the detailed studies this report is the first studying the influence of the amide bond on the geometry of NAC.

The conformations of N-acetyl-L-cysteine-N-methylamide have been analyzed by molecular orbital (MO) calculations [13] and by the restricted Hartree–Fock (RHF) and DFT methodologies [14]. Furthermore the N-acetyl and N-methylamide derivatives of homocysteine [15,16] and selenocysteine [17] and of the normal amino acids tryptophan [18,19] histidine [20], methionine [16], isoleucine [21–23,23], aspartic acid [24–27] and glutamine [28] have been theoretically investigated. A further theoretical study combined with FT-IR and Raman measurements on KBr pellets has been performed on N-acetyl-L-alanine [29], N-acetyl-L-aspartic and glutamic acid [30]. Two-dimensional infrared spectroscopy has been used to investigate the conformations of N-acetyl-L-prolinamide [31]. Matrix-isolation studies have been performed in combination with Fourier transform infrared (FT-IR) and vibration circular dichroism (VCD) on Ac-Gly-NHMe, Ac-L-Ala-NHMe [32] and Ac-L-Pro-NH₂ [33] and with IR on Ac-β-HGly-NHMe, Ac-β-HGly-NHMe [34] and the dipetides dialanine and diglycine [35]. Although the number of theoretical studies on N-acetylated amino acids is increasing only few experimental data are available and for this reason we performed an experimental FT-IR investigation of NAC in an argon matrix.

2. Computational methods

Molecular geometry optimizations and energy calculations were performed at three different levels of theory. Because of their low computational cost, Hartree–Fock (HF) calculations were used for the initial selection. The DFT(B3LYP) level was used for further optimization and frequency analysis of the selected geometries. MP2 optimization and frequency calculations were performed on the most stable geometries found.

DFT methods provide an adequate compromise between the desired chemical accuracy and the computational cost. Although it is well established that some of the weak interactions which are important for amino acids are poorly described by DFT methods, these methods have been successfully used in former studies on amino acids [36–38]. The reason is that interactions due to hydrogen bondings are principally electrostatic and they are reasonably well accounted by DFT or MP2 methods [39].

Valence double- ζ basis sets were used in this work, i.e. 3-21G for the HF method and 6-31G, expanded with diffuse and polarization functions, for the DFT and MP2 methods. The frequencies and the thermodynamical properties were computed with both methodologies. The harmonic DFT vibrations were scaled with variable scaling factors, i.e. 0.95 for $\nu(X-H)$, 0.98 for γ and τ and 0.975 for all other modes. These factors were optimized for this purpose in a former study [40]. Lorentzian line shapes with 2 cm^{-1} full width at half height were used to simulate the theoretical spectra. The potential energy distribution of each fundamental vibrational mode was computed using a non-commercial program designed for this purpose by Dr. L. Lapinski (Institute of Physics, Pol. Acad. of Sciences, Warsaw, Poland) [41]. Relative abundances of the five most stable conformations were estimated using the formula $\Delta G^\circ = -RT \ln K_r$, with ΔG° the difference in Gibbs free energy and K_r the rotamerization constant.

All calculations were performed using the Gaussian 03 ab initio software package [42] on the high performance computing linux cluster of KULeuven.

3. Experimental methods

The matrix-isolation infrared spectra were obtained in the range 3800–500 cm^{-1} by Fourier transformation of 64 interferograms scanned with a Bruker IFS66 spectrophotometer at a resolution of 1 cm^{-1} .

NAC was evaporated in a home-made mini furnace and was co-deposited with a large excess of argon onto a cryogenic CsI window. The temperature of the cold window (16 K) was obtained using an APD Cryogenics closed-cycle helium refrigeration system with an APD 202 6.5 K [43]. The optimal sublimation temperature of monomeric NAC was found to be 323 K in combination with a 2.0×10^{-5} mbar argon pressure in the cryostat. The sublimation temperature of 323 K was large enough to yield matrices with a sufficient concentration of NAC, but still sufficiently low to prevent product decomposition. As a matter of fact, infrared spectra of the solid compound before and after heating in the furnace were compared to evaluate whether product decomposition has occurred.

N-acetyl-L-cysteine (purity > 99%) was purchased from Fluka and was used without further purification. High purity argon gas (99.99990%) was obtained from Air Liquid.

4. Results

The conformational behavior of NAC is determined by seven internal rotational degrees of freedom, as shown in Chart 1. To explore the conformational landscape of NAC, the relevant dihedral angles were systematically varied to generate the input structure for the calculations. For three dihedral angles, i.e. $C_\alpha N-C_\alpha C_{ca}$ (c), $C_\alpha N-C_\alpha C=O$ (d) and $C_{ca} C_\alpha-CS$ (f), the input angle degrees were 0, 60, 120, 180, 240 and 300. The dihedral input angle degrees for $C_\alpha C-SH$ (g) were 0, 90, 180, 270 and for the remaining three dihedral angles $NC_\alpha-C_\alpha C=O$ (b), $OC_\alpha-OH$ (a), $NC_{ac}-CH_3$ (e) the input values were 0, 180. This implies that we initially generated $(6 \times 6 \times 6 \times 4 \times 2 \times 2 \times 2) 6912$ inputs which were decreased to 4043 realistic ones after eliminating structures with overlap or excessive steric hindrance. All these structures were optimized at the HF/3-21G level of theory, which resulted in 438 different geometries with an energy difference smaller than 22 kJ mol^{-1} relative to the most stable structure at the same level of theory. These geometries were then used as starting points for DFT(B3LYP)/6-31++G** calculations, which led to 35 unique conformations with a relative energy difference smaller than 20 kJ mol^{-1} . Vibrational analysis on the DFT level characterized three conformations with one imaginary frequency. The six conformations detected

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