

Anthrax toxin time-resolved absorption and resonance ft-ir and raman biospectroscopy and density functional theory (dft) investigation of vibronic-mode coupling structure in vibrational spectra analysis

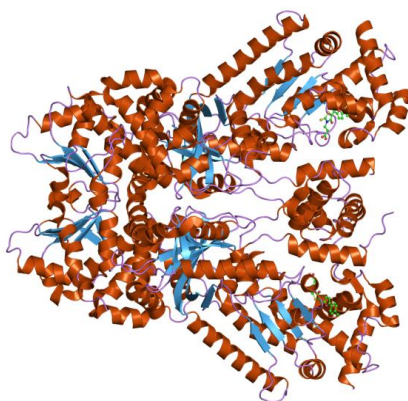
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Abstract

Anthrax toxin is a three-protein exotoxin secreted by virulent strains of the bacterium, *Bacillus anthracis*—the causative agent of anthrax. The toxin was first discovered by Harry Smith in 1954. Anthrax toxin is composed of a cell-binding protein, known as protective antigen (PA), and two enzyme components, called edema factor (EF) and lethal factor (LF). These three protein components act together to impart their physiological effects. Assembled complexes containing the toxin components are endocytosed. In the endosome, the enzymatic components of the toxin translocate into the cytoplasm of a target cell. Once in the cytosol, the enzymatic components of the toxin disrupt various immune cell functions, namely cellular signaling and cell migration. The toxin may even induce cell lysis, as is observed for macrophage cells. Anthrax toxin allows the bacteria to evade the immune system, proliferate, and ultimately kill the host animal. Research on anthrax toxin also provides insight into the generation of macromolecular assemblies, and on protein translocation, pore formation, endocytosis, and other biochemical processes. Parameters such as FT-IR and Raman vibrational wavelengths and intensities for single crystal Anthrax Toxin are calculated using density functional theory and were compared with empirical results. The investigation about vibrational spectrum of cycle dimers in crystal with carboxyl groups from each molecule of acid was shown that it leads to create Hydrogen bonds for adjacent molecules. The current study aimed to investigate the possibility of simulating the empirical values. Analysis of vibrational spectrum of Anthrax Toxin is performed based on theoretical simulation and FT-IR empirical spectrum and Raman empirical spectrum using density functional theory in levels of HF/6-31G^{*}, HF/6-31++G^{***}, MP2/6-31G, MP2/6-31++G^{***}, BLYP/6-31G, BLYP/6-31++G^{***}, B3LYP/6-31G and B3LYP6-31-HEG^{***}. Vibration modes of methylene, carboxyl acid and phenyl cycle are separately investigated. The obtained values confirm high accuracy and validity of results obtained from calculations.



Molecular structure of Anthrax Toxin.

Introduction

Anthrax toxin is a three-protein exotoxin secreted by virulent strains of the bacterium, *Bacillus anthracis*—the causative agent of anthrax. The toxin was first discovered by Harry Smith in 1954. Anthrax toxin is composed of a cell-binding protein, known as protective antigen (PA), and two enzyme components, called edema factor (EF) and lethal factor (LF). These three protein components act together to impart their physiological effects. Assembled complexes

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Key words: vibronic structure, vibrational spectra analysis, density functional theory (dft), anthrax toxin, non-focal functions of becke, correlation functions of lee-yang-parr, time-resolved absorption and resonance, ft-ir and raman biospectroscopy

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containing the toxin components are endocytosed. In the endosome, the enzymatic components of the toxin translocate into the cytoplasm of a target cell. Once in the cytosol, the enzymatic components of the toxin disrupt various immune cell functions, namely cellular signaling and cell migration. The toxin may even induce cell lysis, as is observed for macrophage cells. Anthrax toxin allows the bacteria to evade the immune system, proliferate, and ultimately kill the host animal. Research on anthrax toxin also provides insight into the generation of macromolecular assemblies, and on protein translocation, pore formation, endocytosis, and other biochemical processes. Density Functional Theory (DFT) is one of the most powerful calculation methods for electronic structures [5-7]. Numerous results have been previously studied and indicate successful use of these methods [8-10]. The theory is one of the most appropriate methods for simulating the vibrational wavenumbers, molecular structure as well as total energy. It may be useful to initially consider the calculated results by density functional theory using HF/6-31G', HF/6-31++G'', MP2/6-31G, MP2/6-31++G'', BLYP/6-31G, BLYP/6-31++G'', B3LYP/6-31G and B3LYP6-31-HEG'' approach [11-16]. It should be noted that calculations are performed by considering one degree of quantum interference as well as polarization effects of 2d orbitals in interaction [17-364].

Details of Calculations

All calculations of molecular orbital in the base of ab are performed by Gaussian 09. In calculation process, the structure of Anthrax Toxin molecule (Figure 1) is optimized and FT-IR and Raman wavenumbers are calculated using HF/6-31G', HF/6-31++G'', MP2/6-31G, MP2/6-31++G'', BLYP/6-31G, BLYP/6-31++G'', B3LYP/6-31G and B3LYP6-31-HEG'' base. All optimized structures are adjusted with minimum energy. Harmonic vibrational wavenumbers are calculated using second degree of derivation to adjust convergence on potential surface as good as possible and to evaluate vibrational energies at zero point. In optimized structures

considered in the current study, virtual frequency modes are not observed which indicates that the minimum potential energy surface is correctly chosen. The optimized geometry is calculated by minimizing the energy relative to all geometrical quantities without forcing any constraint on molecular symmetry. Calculations were performed by Gaussian 09. The current calculation is aimed to maximize structural optimization using density functional theory. The calculations of density functional theory are performed by HF/6-31G', HF/6-31++G'', MP2/6-31G, MP2/6-31++G'', BLYP/6-31G, BLYP/6-31++G'', B3LYP/6-31G and B3LYP6-31-HEG'' function in which non-focal functions of Becke and correlation functions of Lee-Yang-Parr beyond the Franck-Condon approximation are used. After completion of optimization process, the second order derivation of energy is calculated as a function of core coordination and is investigated to evaluate whether the structure is accurately minimized. Vibrational frequencies used to simulate spectrums presented in the current study are derived from these second order derivatives. All calculations are performed for room temperature of 434 (K).

Vibration Analysis

Analysis of vibrational spectrum of Anthrax Toxin is performed based on theoretical simulation and FT-IR empirical spectrum and Raman empirical spectrum using density functional theory in levels of HF/6-31G', HF/6-31++G'', MP2/6-31G, MP2/6-31++G'', BLYP/6-31G, BLYP/6-31++G'', B3LYP/6-31G and B3LYP6-31-HEG''. Vibration modes of methylene, carboxyl acid and phenyl cycle are separately investigated.

C-H stretching vibrations in single replacement of benzene cycles are usually seen in band range of 3245-3495 cm^{-1} . Weak Raman bands are at 3234 cm^{-1} and 3247 cm^{-1} . C-C stretching mode is a strong Raman mode at 1244 cm^{-1} . Raman weak band is seen at 1718 cm^{-1} , too. Bending mode of C-H is emerged as a weak mode at 1443 cm^{-1} and 1242 cm^{-1} and a strong band at 1326 cm^{-1} in Raman spectrum. Raman is considerably active in the range of 1245-1495 cm^{-1} which 1248 cm^{-1} indicates this issue.

C-H skew-symmetric stretching mode of methylene group is expected at 3230 cm^{-1} and its symmetric mode is expected at 3044 cm^{-1} . Skew-symmetric stretching mode of CH_2 in Anthrax Toxin has a mode in mid-range of Raman spectrum at 3145-3265 cm^{-1} . When this mode is symmetric, it is at 3140 cm^{-1} and is sharp. The calculated wavenumbers of higher modes are at 3108 cm^{-1} and 3148 cm^{-1} for symmetric and skew-symmetric stretching mode of methylene, respectively.

Scissoring vibrations of CH_2 are usually seen at the range of 1575-1626 cm^{-1} which often includes mid-range bands. Weak bands at 1585 cm^{-1} are scissoring modes of CH_2 in Raman spectrum. Moving vibrations of methylene are usually seen at 1504 cm^{-1} . For the investigated chemical in the current study, these vibrations are at 1384 cm^{-1} were calculated using density functional theory. Twisting and rocking vibrations of CH_2 are seen in Raman spectrum at 960 cm^{-1} and 1334 cm^{-1} , respectively, which are in good accordance with the results at 944 cm^{-1} and 1209 cm^{-1} , respectively.

In a non-ionized carboxyl group (COOH), stretching vibrations of carbonyl [C=O] are mainly observed at the range of 1885-1933 cm^{-1} . If dimer is considered as an intact constituent, two stretching vibrations of carbonyl for symmetric stretching are at 1785-1830 cm^{-1} in Raman spectrum. In the current paper, stretching vibration of carbonyl mode is at 1832 cm^{-1} which is a mid-range value.

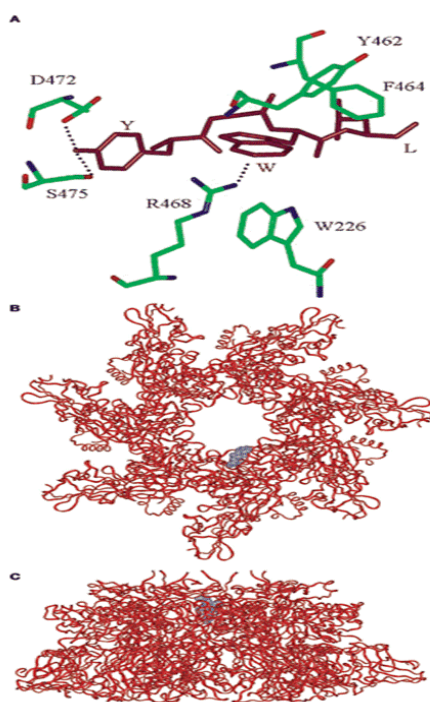


Figure 1. Different sections of the Anthrax Toxin

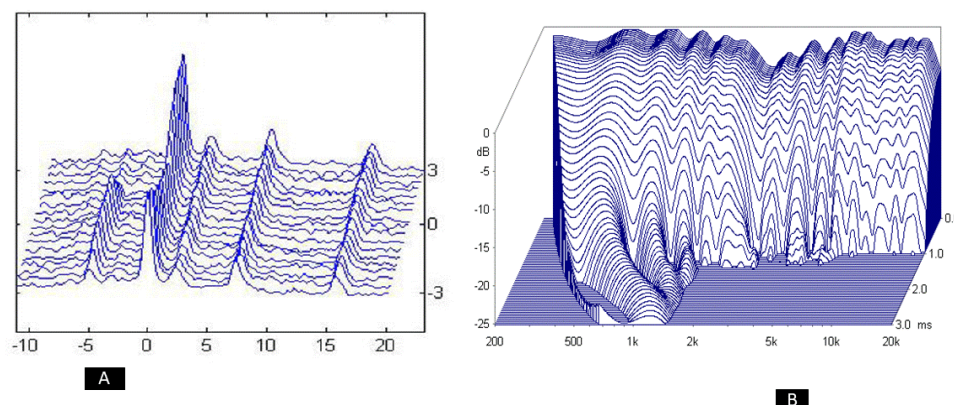


Figure 2. 3D Simulation of (a) FT-IR spectrum and (b) Raman spectrum of Anthrax Toxin

Stretching and bending bands of hydroxyl can be identified by width and band intensity which in turn is dependent on bond length of Hydrogen. In dimer form of Hydrogen bond, stretching band of O-H is of a strong Raman peak at 1412 cm^{-1} which is due to in-plane metamorphosis mode. Out-of-plane mode of O-H group is a very strong mode of peak at 1094 cm^{-1} of Raman spectrum. The stretching mode of C-O (H) emerges as a mid-band of Raman spectrum at 1292 cm^{-1} .

Lattice vibrations are usually seen at the range of $0\text{--}595\text{ cm}^{-1}$. These modes are induced by rotary and transferring vibrations of molecules and vibrations and are including Hydrogen bond. Bands with low wavenumbers of Hydrogen bond vibrations in FT-IR and Raman spectrum (Figure 2) are frequently weak, width and unsymmetrical. Rotary lattice vibrations are frequently stronger than transferring ones. Intra-molecular vibrations with low wavenumbers involving two-bands O-H ...O dimer at 133 cm^{-1} , 238 cm^{-1} and 294 cm^{-1} are attributed to a rotary moving of two molecules involving in-plane rotation of molecules against each other.

Conclusion and Summary

Calculations of density functional theory using HF/6-31G*, HF/6-31++G**, MP2/6-31G, MP2/6-31++G**, BLYP/6-31G, BLYP/6-31++G**, B3LYP/6-31G and B3LYP/6-31-HEG** levels were used to obtain vibrational wavenumbers and intensities in single crystal of Anthrax Toxin. Investigation and consideration of vibrational spectrum confirm the formation of dimer cycles in the investigated crystal with carboxyl groups from each Hydrogen molecule of acid protected from adjacent molecules. The calculated vibrational spectrum which obtains from calculations of density functional theory is in good accordance with recorded empirical values which indicates successful simulation of the problem. The obtained results indicate that the results obtained from theoretical calculations are valid through comparing with empirical recorded results.

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