Characterization of Urease enzyme using Raman and FTIR Spectroscopy

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Abstract: Urease is a commonly found enzyme in the natural biological environment like plants, soil, and animals. Its characteristic decomposition is spectroscopically investigated in acidic (chloride) environment for understanding nitrogen cycle.
OCIS codes: (000.0000) General; (300.0300) Spectroscopy; (170.5660) Raman spectroscopy

1. Introduction

Urease (or urea amidohydrolase) is an enzyme discovered 150 years ago which binds 2 nickel ions per subunit [1]. It is commonly found in unicellular micro-organisms like archaea, eukaryotes, bacteria etc. Being the first enzyme to be crystallized in 1926, it has always been a subject of special attention because it proved the protein nature of enzymes. Additionally, it was the first enzyme to prove the presence of sulfhydryl group along with nickel in a basic trimeric structure with three catalytic centers [2]. The primary nature of urease is to allow active utilization of urea as a nitrogen source. One such example is the extensive use of urea as a nitrogen fertilizer. Urease in plants helps in environmental utilization of these nitrogen compounds based fertilizers and also acts as a catalyzer for seed germination by urea degradation [2]. Apart from the role of urease in plant biochemistry and supporting the nitrogen cycle, the presence of urease carries significant importance in soil chemistry and agricultural practice, for example, recent studies have shown microbes capable of producing urease have biologically mediated metal precipitation by forming carbonates [3]. Biochemical studies of urease crystal show its catalytic nature which hydrolyzes fertilizer urea into ammonia and causes corresponding rise in soil pH level to induce plant damage [4]. Studies have shown similar virulence nature of urease in humans and animals, where infections of urinary and gastrointestinal tracts are observed [5]. The following study, presents a characterization of urease in its natural biological acidic environment [6] using Raman and Fourier Transform Infrared (FTIR) spectroscopy technique in order to further enhance soil productivity.

$$(NH_2)_2CO + H_2O \xrightarrow{Urease} 2NH_3 + CO_2 \quad (1)$$

Since the chemistry of urease reaction is still debatable it is essential to investigate the molecular processes involved in the hydrolysis of urea (shown in equation 1) using spectroscopic techniques. The vibrational fingerprinting of urease in hydrolyzing environment may be useful in establishing its wider role in the natural biological environment by analyzing the evolution of ammonia in the presence of HCl, to represent chloride species in nature. Raman spectroscopy provides symmetric and anti-symmetric vibrations of molecules resulting for polarizability contributions, which may be identifiable from the hydrolysis and chloride exchange reaction in the low to high pH environment, in the presence of Ni^{2+}-ions. In view of the high polarizability of Ni^{2+}-ions in the urease structure may help in identifying the likely changes in the cysteine. FTIR technique (based on infrared absorption molecular spectra), maps out the changes in vibrational spectra (symmetric, asymmetric, antisymmetric, harmonics and overtone bands), arising as a result of hydrolysis in chloride environment.

2.1 Experimental procedure

For detailed vibrational spectroscopic analysis, it was necessary to have baseline spectroscopic studies for analytes and the media: (i) de-ionized water, (ii) diluted urea, (iii) HCl and diluted urea, and (iv) urease mixed with urea, water and HCl, respectively. In each case 0.05M solution was prepared for comparing the data. We used an inVia Renshaw Raman microscope and Bruker ATR-FTIR for the urea and urease samples. The excitation source in inVia Raman microscope was a 514.5 nm laser with laser power of 25 mW at the source, which diminished to below 5mW after the focusing objective on the sample. All the Raman spectra have been corrected by subtracting the background, and deconvoluted the urease Raman spectra using Lorentzian function to identify the exact peak positions. The infrared spectra were recorded on a Bruker Vertex 70 Fourier transform spectrometer, using a MIR source and DTGS detector with a resolution of 4 cm^{-1}.

3. Results and Discussions

Figure 1 a) and b) show the Raman and ATR-FTIR spectrum of pure urea, urease and 0.05M urease solution (mixture of urease, urea, HCl and water). The Raman spectrum of urea has peaks located at 546 cm^{-1}, 1014 cm^{-1}, 1127 cm^{-1}, 1466 cm^{-1}, 1625 cm^{-1} and 1650 cm^{-1} (Fig.1a). The band corresponding to the symmetric CN stretch is experimentally and theoretically reported at, (i) ~1004 cm^{-1} for the solid urea, (ii) 546 cm^{-1} for CO deformation, (iii) 1625 and 1650

cm^{-1} for NH_2 deformation vibrations, (iv) 1538 cm^{-1} for CO symmetric stretching vibration, and (v) 1014 and at 1466 cm^{-1} for the symmetrical and antisymmetric CN stretching vibrations, respectively. Urea is identifiable in the Raman spectrum (Fig.1a) of urease, at 575, 1018, 1075, 1126, 1264, 1365, 1461, 1593, 1635 and 1676 cm^{-1} bands assigned to CN, NH_2 and CO deformation. When comparing the molecular vibrations in urease, the spectra are shifted to lower and higher wavenumbers compared to pure solid urea stretching vibrations, respectively. In the solution spectrum (urease spectrum), the CO stretching vibration is assigned to the band at 1593 cm^{-1}, which appears at a lower wavenumber (1542 cm^{-1}) in the solid urea spectrum. In comparison to NH_2 deformation vibrations bands in Urea (1625 and 1650 cm^{-1}), the vibration bands appear at higher wavenumbers in the solution spectrum (1633 and 1676 cm^{-1}). The Raman band at 1466 cm^{-1} (CN stretching vibrations) in the solid urea spectrum is shifted to a lower wavenumber in solution spectrum (1461 cm^{-1}). The stretching vibrations shift to lower and higher frequencies while moving from the solid spectrum to solution spectrum due to the different bonding character [7]. The vibrational components in urease located at 850 cm^{-1}, 920 cm^{-1}, and 1334 cm^{-1} correspond to HCl, ammonia phosphate and nickel (2+), respectively.

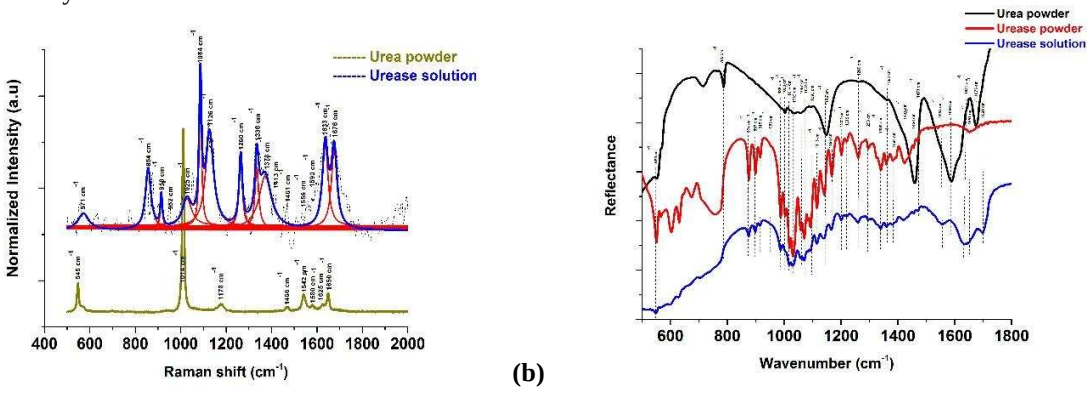

Fig.1 Comparison of a) Raman spectra of urea powder and urease solution, and b) ATR-FTIR spectrum of urea powder, urease powder and urease solution.

Figure 1b shows the ATR-FTIR spectra of urea and urease. The urea and urease show different vibrational bands corresponding to CN, NH_2 and CO stretching modes. The Table 1 shows the comparison of Raman and IR vibrational bands in urease and their assignments. The bands were shifted to lower and higher frequencies corresponding to spectroscopic effects (Table 1) [7]. Prominent peak at 670 cm^{-1} corresponds to presence of nickel. Since the medium of experiments was an aqueous medium of acidic nature. The other peaks corresponds to HCl and water.

Table 1. Raman and Infrared frequencies of urease solution and their assignments.

Raman (cm^{-1})	IR (cm^{-1})	Assignment	Raman (cm^{-1})	IR (cm^{-1})	Assignment
1018-1084	1002-1057	Symmetric CN	1595	1589	Asymmetric NH_2
1126	1145	CO	1635	1615	CO
1466	1457	Asymmetric CN	1676	1685	Symmetric NH_2

4. Conclusions

Detection of urea and urease compounds by using spectroscopic techniques (Raman and ATR-FTIR) is demonstrated. The vibrational bonds in urease by using Raman and ATR-FTIR techniques are different, and stretching vibrations are shifting to lower and higher wavenumber, respectively. The different frequencies and shifting might be due to crystal field splitting and coupling between the CN, NH_2 and CO vibrations.

5. The authors acknowledge the financial support from the GHRG (NiHR-14/137/44) and EU-Marie Curie IIF (BonE-GraphT - REP-752297-1).

6. References

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