Vibrational Spectrum of Gum Agar in Pure and D_2O Exchange state by FTIR Analysis

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Abstract

Gum Agar is an important biomaterial consists of a mixture of agarose and agaropectin. Agarose is a linear polymer. In this work Fourier transformed infrared (FTIR) absorption and Attenuated total reflectance (ATR) spectrum of Gum Agar samples at different polymerized state with H_2O and D_2O are analyzed and compared. The changes in characteristics bond vibration due to environmental variation are detected clearly. The obtained shift of the characteristics frequencies and absence of some peaks are caused due to change in molecular structure in the types of specimens.

1. Introduction

Agar is an important biomaterial consists of a mixture of agarose and agaropectin. Agarose is a linear polymer, of molecular weight about 120,000. An agarose is a polysaccharide polymer material, generally extracted from seaweed. Agarose is a linear polymer made up of the repeating unit of agarobiose, which is a disaccharide made up of D-galactose and 3, 6-anhydro-L-galactopyranose. Agarose is frequently used in molecular biology for the separation of large molecules, especially DNA, by electrophoresis. On the other hand agaropectin is a heterogeneous mixture of smaller molecules that occur in lesser amounts. Their structures are similar but slightly branched and sulfated, and they may have methyl and pyruvic acid ketal substituents. Gum Agar has a poor gel formation ability compared to that with agarose alone. Agar is insoluble in cold water but dissolves to give random coils in boiling water.

FTIR spectroscopy is a measurement of variation intensity of the absorption of IR radiation by a sample with wavelength of IR radiation [1]. This work makes an attempt to detect structural change in Gum Agar samples at different polymerized state with H_2O and D_2O by FTIR spectroscopy.

There are some proteins of alike structure are present in agar. The amide I and II bands are the two most prominent vibrational bands of the protein backbone. The frequencies of the amide I band components are found to be correlated closely to the each secondary structural element of the proteins. The amide II band, in contrast, derives mainly from in plane N-H bending (40-60% of the potential energy) and from the CN stretching vibration (18-40%), showing much less protein conformational sensitivity than its amide I counterpart. Other amide vibrational bands are very complex depending on the details of the force field, the nature of side chains and hydrogen bonding, which therefore are of little practical use in the protein conformational studies [2]. N-H bending, N-H stretching, C-N stretching vibrations are found in Agar spectrum. The overall results give a good account of change in molecular structure in the Gum Agar specimens.

2. Sample

The Gum Agar powder specimen (S1) was collected from Merk (India) and was subjected to a sol–gel process along with (i) sterile water and (ii) D_2O. In both the polysaccharide host chain can form more complex higher polymers over that of its normal powdered form. D_2O exchange can occur leading to replacement of -OH group by -OD. The sol specimens are extracted at initial state, 30, 60 and 120 minutes after that. The experimental specimens S2-S5 (sol specimens of D_2O) were developed with subsequent adequate drying of the sols at environmental condition.

3. Experiments

The developed Gum Agar specimens are supposed to exhibit change in molecular structure over that in S1 due prolongation of sol gel process. FTIR analysis on pure Gum Agar and specimens S2 to S5 were carried out to examine its molecular structure and dynamical information. The analysis was carried out using FTIR model, IR affinity1, Shimadzu, Japan, at high resolution (resolution was 0.5 cm^{-1}) using KBr window. The sol specimens of Gum Agar with H_2O and D_2O collected at 0, 30, 60 and 120 minutes after sol initiation and were also analyzed by ATR using ZnSe window at 0.5 cm^{-1} resolution.

4. Results & Discussions

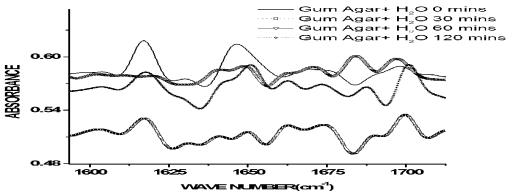

Figure 1. ATR absorption spectrum of different Gum Agar specimens in between 1595 to 1710 cm^{-1} at 25ºC

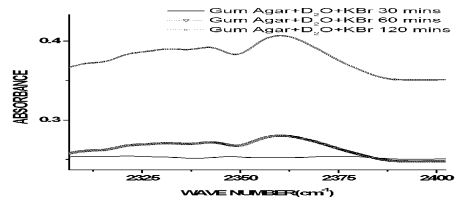

Figure 2. FTIR absorption spectrum of different Gum Agar specimens in between 2315 to 2400 cm^{-1}at 25ºC.

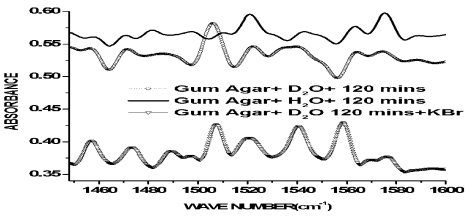

Figure. 3. FTIR and ATR absorption spectrum of different Gum Agar specimens in N-H bend region (between 1450 to 1600 cm^{-1}) at 25ºC.

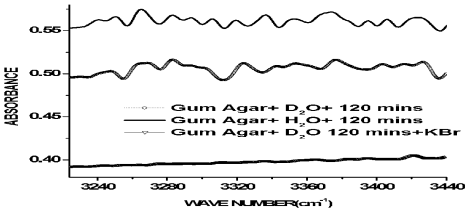

Figure. 4. FTIR and ATR absorption spectrum of different Gum Agar specimens in N-H stretch region (between 3225 to 3440 cm^{-1}) at 25ºC.

Fig. 1 shows the comparison of the ATR spectrum of different Gum Agar sol specimens between wave number 1595 to 1710 cm^{-1}. The result gives a clear picture for change in molecular structure in the bio-molecule due to polymerization. In Fig. 2 FTIR spectrum (between wave number 2315 to 2400 cm^{-1}) of Gum Agar specimens (sol specimens of D_2O) developed by adequate drying of the sols at environmental condition are compared. From this D_2O peaks are clearly observed for the specimens collected 60 and 120 minutes after initial state but is absent for the specimen collected 30 minutes after initial state. Both the FTIR and ATR spectrum are compared in Fig. 3 and Fig. 4 as the solid samples of Gum Agar were used besides the liquid specimens. Comparison of N-H bending of different specimens is shown in Fig. 3 between wave number 1450 to 1600 cm^{-1}. Fig. 4 shows the comparison of the spectrum of different Gum Agar specimens between wave number 3225 to 3440 cm^{-1} in N-H stretching region. The N-H peaks are often very broad and weak without any distinct coupling to hydrogen's on an adjacent carbon atom. This condition can be caused by chemical exchange of the –NH proton or by a property of nitrogen atoms called quadrupolar broadening. The amino hydrogens will exchange with D_2O, causing the peaks to disappear [3].

$$\text{-N-H} + D_2O = \text{-N-D} + \text{DOH} \qquad (1)$$

Fig. 3 and Fig. 4 indicate that some –N-H peaks are absent from the spectra due to D_2O exchange as shown in Eq. (1).

5. Conclusions

The change in molecular structure, of the constituent bio-molecules of Gum Agar, due polymerization is detected from analysis of FTIR and ATR spectra. The distinction in molecular vibration due to the D_2O exchange is also clearly visible. IR absorbance of D_2O exchanged dried Agar specimens show an increase in peak height with increase of sol-gel time. Due to D_2O exchange -NH bending and stretching peaks disappeared from the spectra. This study also confirms that rehydration occur only for the –NH bending but structural change occurred in –NH stretching are permanent and no rehydration takes place.

6. Acknowledgement

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7. References

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