



Chemical composition of amylase determined by IR spectroscopy

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Abstract

In this article, we determined the chemical composition of amylase by IR spectroscopy using a PerkinElmer SpectrumOne spectrometer. The wavelength range in which absorption bands appear is between 4500cm^{-1} and 400cm^{-1} . Amylase contains the following functional groups: Ag-O, N-H, O-H, C-O. Infrared (IR) spectroscopy was utilized to investigate the chemical composition and structural characteristics of the enzyme amylase. The IR spectrum exhibited distinct absorption bands corresponding to the functional groups typical of protein molecules. Prominent peaks were observed around 3300cm^{-1} (N-H and O-H stretching), 1650cm^{-1} (amide I, C=O stretching of peptide bonds), and 1540cm^{-1} (amide II, N-H bending and C-N stretching). These characteristic protein bands confirm the presence of peptide linkages as the major structural feature of amylase. Minor peaks associated with side-chain functional groups further supported the complex tertiary structure of the enzyme. Overall, the analysis demonstrates that IR spectroscopy is an effective and non-destructive analytical technique for identifying the molecular composition and confirming the proteinaceous nature of amylase.

Keywords: Chemical composition, amylase, IR spectroscopy

Introduction

Amylose is a polysaccharide made of α -D-glucose units, bonded to each other through $\alpha(1\rightarrow4)$ glycosidic bonds. It is one of the two components of starch, making up approximately 20–30%. Because of its tightly packed helical structure, amylose is more resistant to digestion than other starch molecules and is therefore an important form of resistant starch.

Amylose is made up of $\alpha(1\rightarrow4)$ bound glucose molecules. The carbon atoms on glucose are numbered, starting at the aldehyde (C=O) carbon, so, in amylose, the 1-carbon on one glucose molecule is linked to the 4-carbon on the next glucose molecule ($\alpha(1\rightarrow4)$ bonds). The structural formula of amylose is pictured at right. The number of repeated glucose subunits (n) is usually in the range of 300 to 3000, but can be many thousands [1-5].

There are three main forms of amylose chains can take. It can exist in a disordered amorphous conformation or two different helical forms. It can bind with itself in a double helix (A or B form), or it can bind with another hydrophobic guest molecule such as iodine, a fatty acid, or an aromatic compound. This is known as the V form and is how amylopectin binds to amylose in the structure of starch. Within this group, there are many different variations. Each is notated with V and then a subscript indicating the number of glucose units per turn. The most common is the V6 form, which has six glucose units a turn, [4] V8 and possibly V7 forms exist as well. These provide an even larger space for the guest molecule to bind.

This linear structure can have some rotation around the phi and psi angles, but for the most part bound glucose ring oxygens lie on one side of the structure. The $\alpha(1\rightarrow4)$ structure promotes the formation of a helix structure, making it possible for hydrogen bonds to form between the oxygen atoms bound at the 2-carbon of one glucose molecule and the 3-carbon of the next glucose molecule.

Fiber X-ray diffraction analysis coupled with computer-based structure refinement has found A-, B-, and C-

polymorphs of amylose. Each form corresponds to either the A-, the B-, or the C- starch forms. A- and B- structures have different helical crystal structures and water contents, whereas the C- structure is a mixture of A- and B- unit cells, resulting in an intermediate packing density between the two forms [6-12].

Materials and Methods

FT-IR spectra were recorded on PerkinElmer SpectrumOne spectrometer, operated at a resolution of 1.0cm^{-1} with KBr and mylar beam splitter. Pure amylase the spectra were read between 4500 and 400cm^{-1} with a resolution of 1cm^{-1} . The spectra of the samples were recorded thrice and averaged to ensure reproducibility. All the spectral measurements were performed at $25\pm 3\text{ }^{\circ}\text{C}$.

FTIR spectra revealed the characteristic peak shifts in the test sample of interacted amylase with AgNPs. The binding of amylase with AgNPs was confirmed by FTIR analysis. The interacted sample exhibited peaks at 500 , 517 and 528cm^{-1} which is possibly assigned to the Ag-O bonds (Fig. 2). Amide I region is based on the stretching vibrations of C- O and N-H of the amide group. The bands obtained at 3500cm^{-1} and between 1600 and 1700cm^{-1} indicated the presence of amide groups upon adsorption onto AgNPs. The shift in the peak position on interaction with AgNPs represents the change in protein structure from its native form. An intense peak at 1645cm^{-1} assigned to the bending vibrations of the N-H group resulted in a shift with the peak to 1738cm^{-1} . There were other characteristic bands at 1168 and 1033cm^{-1} which could be of the stretches and bends of aliphatic amines. An intense absorption peak at 1216cm^{-1} (Fig. 2) was due to interaction of the NH group of amylase with the Ag-O group of NPs. [8-16] The strong and broad peak at 3400cm^{-1} (Fig. 2) can be attributed to the O-H vibration on the sample surface, [17] The band at 1435cm^{-1} is due to the N-H stretching vibrations present in the amide linkages of the proteins, [18]



Fig 1: PerkinElmer SpectrumOne spectrometer

Results and discussions

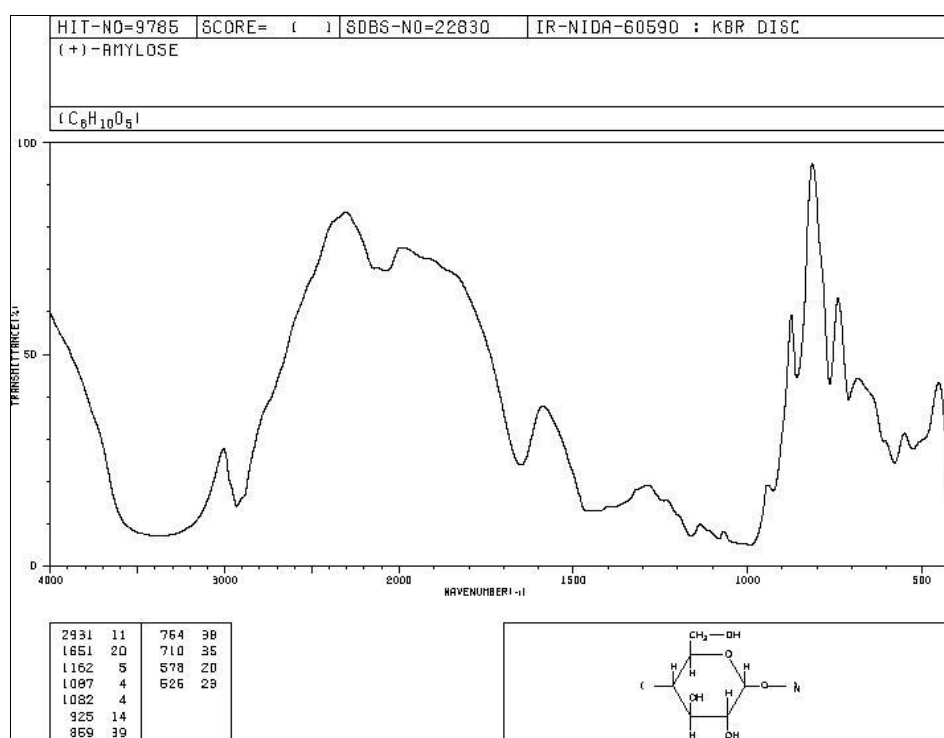


Fig 2: Spectrul IR al amilazei

Conclusions

The structural formula of amylose is pictured at right. The number of repeated glucose subunits (n) is usually in the range of 300 to 3000, but can be many thousands. Amilazei cuprinde urmatoarele grupe functionale: Ag-O, N-H, O-H, C-O intre urmatoarele lungimi de unda 4500 cm⁻¹si 400cm⁻¹. The IR spectroscopic analysis confirmed that amylase is a protein-based compound composed primarily of peptide bonds and characteristic amino acid functional groups.

The presence of amide I and amide II absorption bands verified the polypeptide backbone structure typical of enzymes. Additional peaks indicated the presence of hydroxyl and carboxyl groups from amino acid side chains, reflecting the complex tertiary structure of the molecule. These findings demonstrate that IR spectroscopy is a reliable and non-destructive method for identifying the chemical composition and verifying the protein nature of amylase.

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