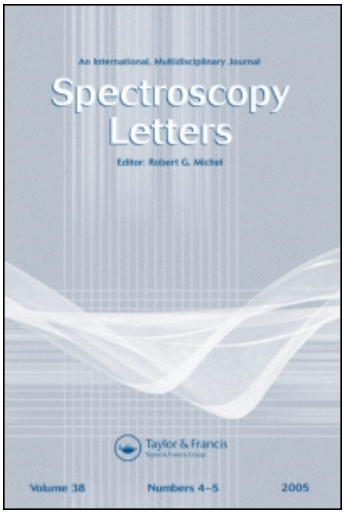

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

ENZYMES AS STRUCTURAL TOOL IN INFRARED SPECTROSCOPY

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Online publication date: 14 August 2002

To cite this Article Offmann, Bernard and Cadet, Frédéric(2002) 'ENZYMES AS STRUCTURAL TOOL IN INFRARED SPECTROSCOPY', Spectroscopy Letters, 35: 4, 523 — 526
To link to this Article: DOI: 10.1081/SL-120013888
URL: http://dx.doi.org/10.1081/SL-120013888

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SPECTROSCOPY LETTERS, 35(4), 523–526 (2002)

ENZYMES AS STRUCTURAL TOOL IN INFRARED SPECTROSCOPY

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ABSTRACT

The complexity of IR spectra of some compounds, particularly biological molecules, is a major obstacle in assigning or characterizing their IR bands. In this short communication, the potential use of enzymes for infrared bands characterization and assignments is demonstrated and discussed.

Key Words: Enzyme; ATR; Mid-infrared spectroscopy

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INTRODUCTION

The literature on chemical assignments for infrared bands is summarized in the form of a spectra-structure correlation diagram and as a list of assignments arranged in order of increasing wavelength. Although these summaries represent only some of the most important IR bands, the degree of complexity of spectra is obvious. It may be necessary to check the identity of chemical groups in a molecule by the use of characteristic chemical or derived assays.

In this note, the use of enzymes in infrared spectroscopy, as an alternative method for verification and/or for assignment of bands, is proposed. The characteristic property of enzymes is their power of catalyzing certain specific definite chemical reactions. Several thousand enzymes-catalyzed reactions are known. This information can be very useful in infrared spectroscopy. The assignment of the band corresponding to the disaccharide link of sucrose is developed as an example. In this study, an attenuated total reflectance (ATR) cell is used instead of the ordinary transmission cell.

EXPERIMENTAL METHODS

70 μL of yeast β-fructosidase (124 μg) are added, at $t = 0$, to 7 mL of 20% (m/v) sucrose solution in citrate-phosphate 0.1 M buffer pH 6. Mid-FTIR transformed spectra are collected at fixed cycle time (45 s), using a macro command (experiment could be carried out with a chronometer), on a Bomem-Michelson MB-100 Fourier transformed spectrophotometer. Attenuated total reflectance spectra are obtained with a Specac Overhead ATR System. The crystal of the reflectance element is made from zinc selenide, a material that is quite inert to water.

The data were collected from 800 to 1250 cm^{-1} in 4 cm^{-1} increments as log(1/R), in which R is the ratio of the reflected intensity for the background to that of the sample. Although the ATR experiment does involve the reflection of the radiation within a crystal, the interaction of the radiation with the sample is a transmittance of radiation though the sample; this depth of penetration is wavelength dependant, but it is still passing through a finite layer of the sample. For this reason, plots can be read according to absorbance (or transmittance).

RESULTS AND DISCUSSION

Sucrose is a disaccharide made up of α-D-gluco-pyranosyl and β-D-fructofuranoside groups. Major differences between the infrared spectra of

sucrose and an α-D-glucose/β-D-fructose mixture are exhibited at about 996 cm^{-1}. This broad band, only present in the spectrum of sucrose, suggests that it could be assigned to the vibrational motion of the glycosidic link (1α-2β).

This assignment has already been established by acidic hydrolysis of this sugar,[1] but, this method is neither convenient nor reliable : hydrolysis is carried out for several hours, variations in absorbance are very weak and the employed acid concentration also induces rapid mutarotation of the D-monosaccharides liberated (equilibration between α-glucose and β-glucose and between α and β-fructose). We present here an alternative method of verification that utilizes an enzyme (yeast β-fructosidase, EC 3.2.1.26).

During enzymatic hydrolysis of sucrose, products appear according to:

$$\text{Sucrose} \xrightarrow{\beta-\text{fructosidase}} \alpha\text{-D-glucose} + \beta\text{-D-fructose} \qquad \text{(a)}$$

With the use of this specific enzyme, reaction is faster than the one by acidic hydrolysis and assignment of bands unambiguous. The total reaction

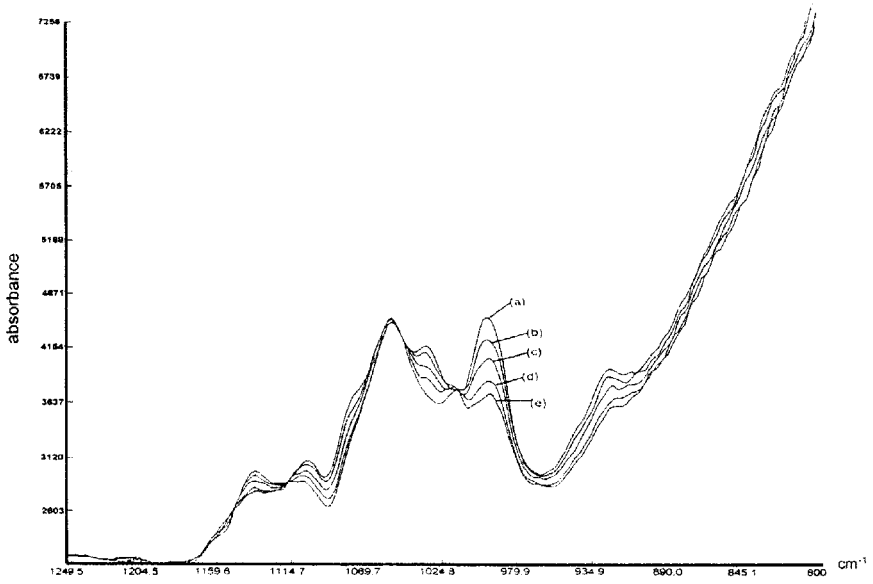

Figure 1. Mid-infrared assignment of disaccharide link as shown by the hydrolysis of sucrose by yeast β-fructosidase. Shown are ATR FTIR spectra observed during the enzymatic hydrolysis of sucrose in aqueous solution. (a) before enzyme addition, t = 0 min; (b) 5 min after enzyme addition; (c) 10 min after enzyme addition; (d) 15 min after enzyme addition; (e) 20 min after enzyme addition.

time depends on enzyme concentration added. In Fig. 1, reaction was performed in twenty minutes at pH 6. It can be quicker ; high enzyme concentration allows quasi-instantaneous reaction and structural modifications. The cleavage of the disaccharide link is clearly exhibited at 996 cm^{-1}. Absorbance augmentation at other wavelengths, essentially at 1033 and 1083 cm^{-1}, are related to the appearance of glucose and fructose. It is nowwell established that this band cannot be associated with the mutarotation of liberated D-oses induced by the employed acid concentration. It can be shown that spectral changes result solely from the hydrolysis of the disaccharide link.

Thus, the FTIR spectroscopy coupled with the use of specific enenzymes will prove of immense help in solving structural problems.

Significant results have been obtained with polyphenol oxidase (unpublished) for the oxidation of phenolic substrate. Futhermore, we have investigated the potentialities of mid-infrared spectroscopy combined with multidimensional statistical analysis to allow one step enzymatic assays.[2,3]

ACKNOWLEDGMENTS

This work was supported by a grant from the Ministère de l'Enseignement Supérieur et de la Recherche.

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Received September 6, 2001
Accepted April 29, 2002