Monatshefte für Chemie 120, 343—348 (1989)

Monatshefte für Chemie
Chemical Monthly

Kinetic Method for the Study of Xylan Hydrolysis by Xylan Hydrolases

Nadka G. Bakalova, Methodi L. Chetkarov, Todor K. Nikolov, and **Dimiter N. Kolev***

Faculty of Biology, University of Sofia, 1421 Sofia, Bulgaria

Summary. The Somogyi-Nelson colorimetric method was used in a new manner more suitable for evaluating the kinetics of the enzyme hydrolysis of xylan catalyzed by xylan hydrolases. The values of the Michaelis parameters ($Km = 5.56\,g\,l^{-1}$ and $V = 2.94 \cdot 10^{-5}\,M\,s^{-1}$) were determined.

Keywords. Xylan hydrolases; Xylan hydrolases kinetics; Endo-1,4-β-xylanase; Xylan 1,4-β-xylosidase.

Eine kinetische Methode zur Untersuchung der Hydrolyse von Xylan durch Xylan-Hydrolase

Zusammenfassung. Die kolorimetrische Methode nach Somogyi-Nelson wurde nach einem neuen Verfahren zur Verfolgung der Kinetik der hydrolytischen Spaltung von Xylan, katalysiert durch Xylan-Hydrolasen von *Aspergillus oryzae,* angewandt. Es wurden die Michaelis-Parameter ($Km = 5.56\,g\,l^{-1}$ und $V = 2.94 \cdot 10^{-5}\,M\,s^{-1}$) bestimmt.

Introduction

The plant raw materials contain cellulose, hemicellulose (often xylan) and lignin. The biodegradation usually includes enzymatic hydrolysis of either or both of xylan and cellulose. The hydrolysis of xylan may be a separate process (e.g. for the production of xylose or xylitol) or simultaneous with ethanol fermentation (e.g. the pentoses derived from initial acid or enzymatic hydrolysis of xylan may be fermented to ethanol with *Fusarium* molds or *Pachysolen* yeasts) [1, 2]. The xylan hydrolases: *endo*-1,4-β-xylanase (EC 3.2.1.8) and xylan 1,4-β-xylosidase (EC 3.2.1.37) catalyze the hydrolysis of the 1,4-β-*D*-xylosidic linkages in xylans to *D*-xylose [3].

In our preceding papers [4, 5] we described the kinetics of the cellobiose hydrolysis catalyzed by β-glucosidase and sodium carboxymethylcellulose hydrolysis catalyzed by a cellulase complex. In this paper we describe the kinetics of xylan hydrolysis, catalyzed by xylan hydrolases.

Experimental

Enzyme Source

As a source of the xylan hydrolases the commercial cellulase preparation "Luizym"® from *Aspergillus oryzae* (Luitpoldwerk, Federal Republic of Germany) was used, which contains cellulase enzymes.

Substrate

As substrate was used xylan ex larch sawdust (Koch-Light Laboratories Ltd., England).

Chemicals

The chemicals used in this work were purchased from Koch-Light Laboratories Ltd., England.

Measurement of Reducing Power

The reducing power of the D-xylose was determined by the unmodified Somogyi-Nelson method [4].

Kinetic Measurements

The enzyme-substrate reaction was carried out in a 1 cm cell (total volume 2.5 ml) in the recording two-beam spectrophotometer "Specord UV-VIS" (Carl Zeiss, German Democratic Republic) at $pH = 4.0$ (0.1 M acetate buffer) and a temperature of 38 °C. Right at the beginning of the reaction a colour reagent (0.05 ml, 2% of the total volume) consisting of solutions of Somogyi's (A : B = 25 : 1) and Nelson's reagents [4] in a volume ratio 1 : 1, was added to a mixture of a buffer solution (0.1 M acetate buffer, $pH = 4.0$), xylan and an enzyme preparation. In control experiments this composition was retained with the enzyme omitted. The continuous change in the absorption of the sample was automatically recorded at 775 nm at different time intervals ($0 \leqslant t \leqslant 90$ min). The time required to record a single curve was 1 min.

Results and Discussion

The recorded absorption spectra from eight *D*-xylose solutions obtained by the unmodified method of Somogyi-Nelson are presented in Fig. 1. The relation between the absorbance change (ΔA) at 775 nm and the concentrations of *D*-xylose is shown in Fig. 2. The molar absorption coefficient (ε) determined from this figure is $\varepsilon_{775} = 2.08 \cdot 10^4$ ($M^{-1}\,\mathrm{cm}^{-1}$).

Fig. 3 represents the absorption spectra of solutions at various times ($0 \leqslant t \leqslant 90$ min) after the beginning of the reaction. These solutions were obtained by mixing xylan hydrolases, xylan and the colour reagent. The change in the absorbance (ΔA), determined at 775 nm, is

$$\Delta A = A_t - A_0 = \varepsilon [P]_t d,$$

where A_0 and A_t are the absorbance of a given solution at an initial and an arbitrary moment of the reaction time; d is the length of the optical path in centimeters; ε is the molar absorption coefficient, and $[P]_t$ is the concentration of the accumulated reaction product (*D*-xylose).

The relation between the change in absorbance at 775 nm and the reaction time (0–5 h) during the hydrolysis of xylan catalyzed by the xylan hydrolases, with different concentrations $[E]_0$ of the latter, is shown in Fig. 4. At increased concentrations of the enzyme the kinetic curves show a clear two step behaviour, corresponding probably to the effect of the different xylan hydrolases: *endo*-1,4-

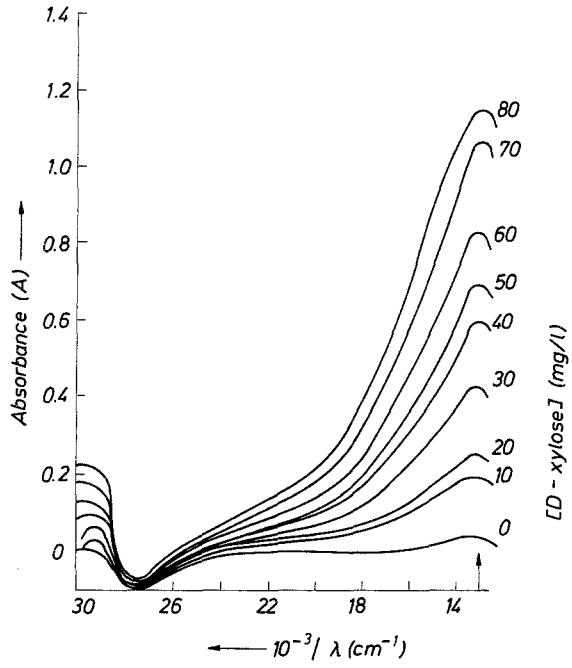

Fig. 1. Absorption spectra of the solutions of *D*-xylose (10 to 80 μg ml^{-1}) using the unmodified method of Somogyi-Nelson

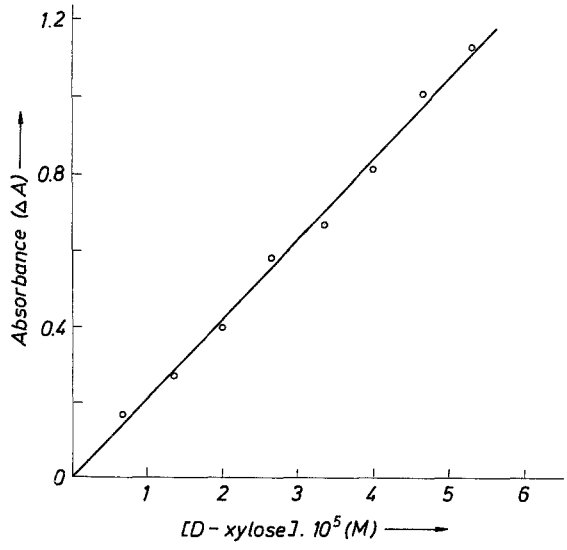

Fig. 2. Calibration curve of *D*-xylose determined by the unmodified Somogyi-Nelson method at 775 nm

β-xylanase (*endo*-acting) and xylan 1,4-β-xylosidase (*exo*-acting). In our preceding paper [5] we found that the kinetic curves of the cellulase enzyme complex show a clear three step behaviour, corresponding to the effect of the different components of the complex.

The initial reaction rates (v_0) were determined from the kinetic curves (Fig. 5), obtained at different concentrations of substrate $[S]_0$ and at a constant enzyme $[E]_0$ concentration.

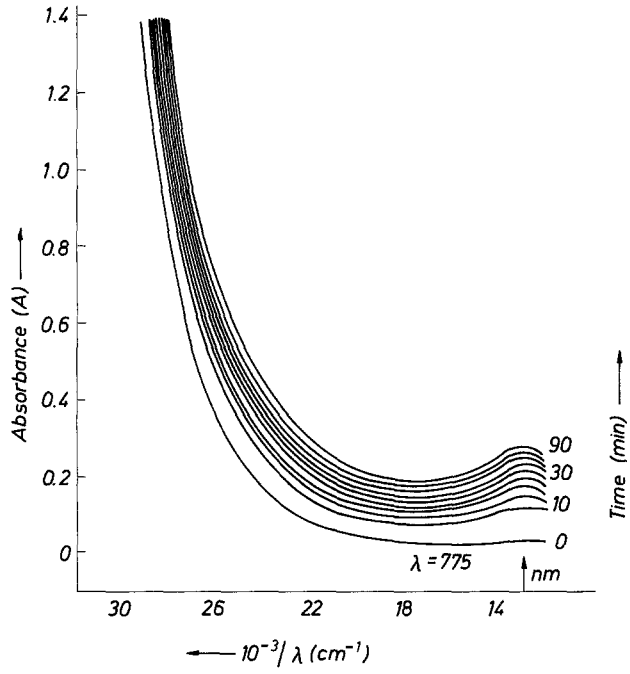

Fig. 3. Absorption spectra of the reaction mixture of xylan hydrolases ($0.75\,g\,l^{-1}$) from *Aspergillus oryzae*, xylan ($8\,g\,l^{-1}$) and colour reagent (0.05 ml) at various times (0 to 90 min), $pH = 4.0$ and 38°C, using the suggested method

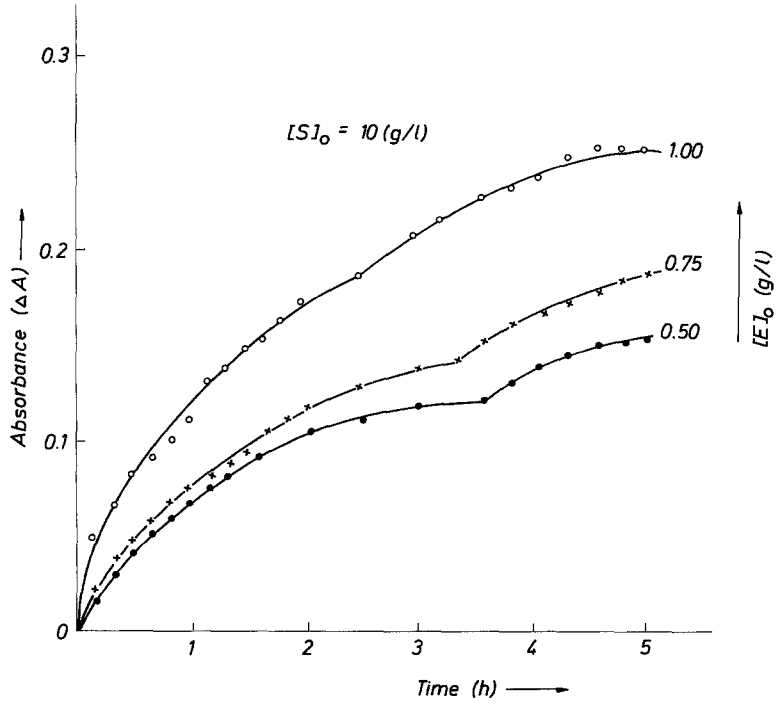

Fig. 4. The step kinetic curves of the reaction "xylan–xylan hydrolases" at different enzyme concentrations (0.50, 0.75 and $1.0\,g\,l^{-1}$) and a constant substrate concentration ($10\,g\,l^{-1}$)

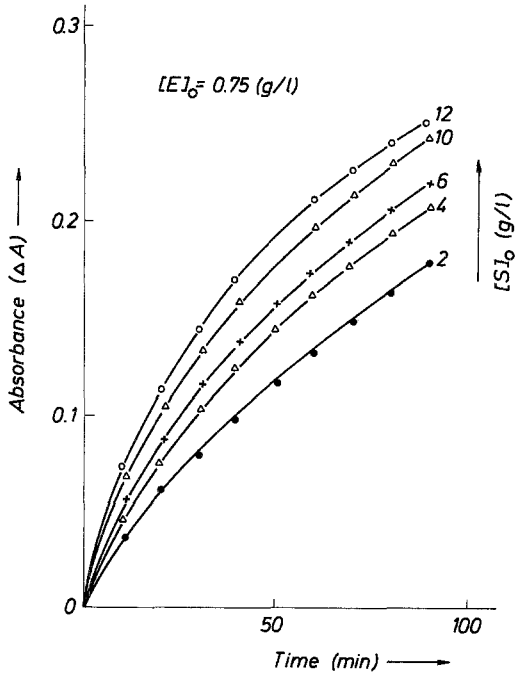

Fig. 5. Kinetic curves of the reaction "xylan – xylan hydrolases" at different substrate concentrations (2 to 12 $g l^{-1}$) and a constant enzyme concentration (0.75 $g l^{-1}$)

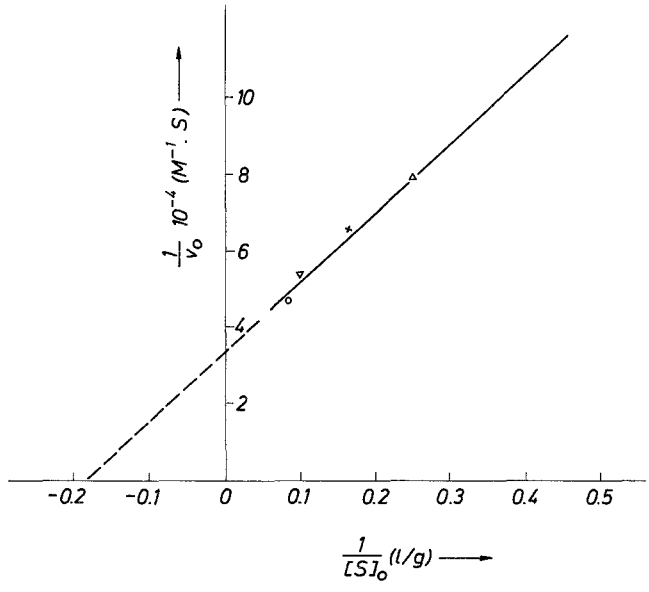

Fig. 6. Lineweaver-Burk plot for xylan hydrolases from *Aspergillus oryzae*

Fig. 6 shows a Lineweaver-Burk [6] plot for xylan hydrolases from *Aspergillus oryzae*. The values of the Michaelis parameters are $Km = 5.56\,g l^{-1}$ and $V = 2.94 \cdot 10^{-5} M s^{-1}$.

The proposed new method excludes any inhibition of the product, due to continuous removal of the latter by the colour reagent. The method does not require heating and subsequent cooling. It is easily reproduced, simple and demands a relatively short time to obtain a kinetic curve. The error is smaller than 8%.

References

[1] Rodionova N. A., Gorbachyova I.-M. V., Tavobilov I. M. (1986) In: Skrijabin G. K., Golovlev E. L., Klyosov A. A. (eds.) Problems of the Bioconversion of Plant Raw Materials (in Russian). Nauka, Moscow, p. 237
[2] Linko M., Rättö M., Viikari L., Bailey M. (1983) In: Duckworth H. E. (ed.) Int. Symp. Ethanol from Biomass. The Royal Society of Canada, Winnipeg, p. 371
[3] IUB (1984) Enzyme Nomenclature. Academic Press, Orlando FL
[4] Chetkarov M. L., Hatour F. D., Kolev D. N. (1984) Monatsh. Chem. **115**: 1321
[5] Chetkarov M. L., Hatour F. D., Kolev D. N. (1985) Monatsh. Chem. **116**: 1433
[6] Lineweaver H., Burk D. (1934) J. Am. Chem. Soc. **56**: 658

Received October 5, 1987. Accepted November 11, 1987