

A Simple and Rapid Determination of Alginate Lyase Activity

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A simple and rapid method for determination of alginate lyase activity has been investigated only by following the increase in absorbance in ultraviolet region of the reaction mixture.

Partially purified enzyme from wreath shell (*Turbo cornutus*) acted on alginic acid, giving good linearity of the absorbance at 235 nm against the reaction time up to about 5 min just after the initiation of the enzyme reaction under the condition applied. The initial reaction velocity can be easily obtained from the straight line in a simple operation.

Alginate lyase [EC 4.2.99.4] acts on alginic acid, which is suggested to consist of mannuronic and guluronic acid,¹⁻³⁾ liberating uronides and uronic acid monomers with reducing end group and double bond on C₄-C₅ linkage of a pyranose ring at the site of the action.⁴⁻¹⁰⁾

Based on the reducing power and the spectrophotometric or hydrodynamic properties of the reaction products, various methods for determination of alginate lyase activity have been reported. These methods are laborious, time-consuming and less useful for general kinetic analysis of enzyme action.

In the experiments described in this paper, a simple and rapid method has been investigated only by following the increase in absorbance in ultraviolet region originated in formation of the double bond (eliminase action). Similar methods have been reported by Nakada *et al.*,¹¹⁾ Preiss and Ashwell⁶⁾ and Nakada and Sweeny⁹⁾ for investigation of eliminase.

MATERIALS AND METHODS

MATERIALS

Alginic acid was purchased from Wako Pure Chemical Co. Tris (hydroxymethyl) aminomethane was a product of Sigma Chemical Co. SP-Sephadex C-50 was a product of Pharmacia Co. Ammonium sulfate (SP grade) was obtained from Nakarai Chemicals Ltd.

METHODS

Preparation of alginate lyase. The mid-gut gland of wreath shell (*Turbo cornu-*

tus) was homogenized with 0.05 M phosphate buffer (pH 6.0) and was left overnight at 5°. After removal of insoluble matters by centrifugation, the fraction of the supernatant treated with ammonium sulfate (50-100%) was dialysed against several changes of 0.01 M phosphate buffer (pH 6.0) and applied onto a top of a column of SP-Sephadex C-50 equilibrated previously with the same buffer. Most active fractions eluted with 0.05 M phosphate buffer (pH 7.0) were pooled, concentrated on Diaflo membrane (UM-10) after the pH of the solution was adjusted to about 6 with dilute phosphoric acid to avoid the enzyme inactivation,* and used for the experiments after dilution with 0.01 M phosphate buffer (pH 6.0) just before use (see figures in detail).

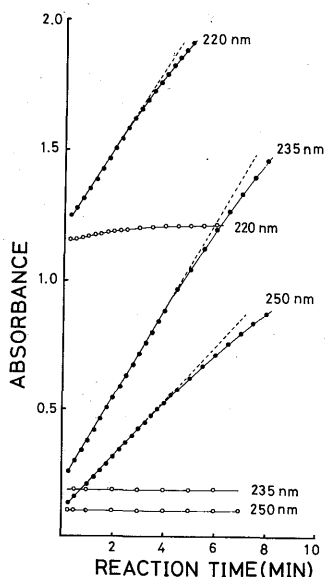


Fig. 1. Time course of the absorbance at different wavelengths of the reaction mixture.

The reaction mixture contained 2.0 ml of 0.1% alginic acid in 0.1 M Tris-HCl buffer (pH 8.3) and 0.2 ml of the enzyme solution (●-●) or 0.2 ml of 0.01 M buffer phosphate (pH 6.0) (○-○).

Readings of the absorbance were carried out as described in text.

Assay of alginate lyase. The reaction was initiated by adding 0.2 ml of the enzyme solution to 2.0 ml of alginic acid in 0.1 M Tris-HCl buffer (pH 8.3) preincubated at 30° for 5 min. ** After mixing the reaction mixture and transferring it in quartz cuvette with 1-cm light pass promptly, readings of the absorbance were taken at time intervals of 15 seconds against a water blank in a Beckman-Toshiba DSB-70 spectrophotometer with a cell compartment attached to a temperature-constant water bath (30°).

RESULTS AND DISCUSSION

Changes in the absorbance at 220, 235 and 250 nm of the reaction mixture under the reaction conditions were found to be linear up to at least 3 min after addition of the enzyme as shown in Fig. 1. The absorbance at 220 nm of the substrate solution was much higher, and its intensity increased spontaneously though in a minute degree when compared to the others. The increase in the absorbance per minute (initial reaction velocity) obtained from the straight line in the figure was 0.160 at 220 nm, 0.170 at 235 nm and 0.108 at 250 nm.

Fig. 2 shows the time course of the absorbance at 235 nm in different enzyme con-

* Unpublished observations

** Optimal activity for this enzyme was obtained at pH 8.8 to 9.2

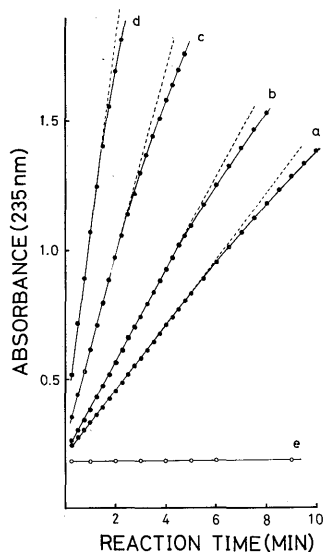


Fig. 2. Time course of the absorbance at 235 nm in the different enzyme concentrations.

The reaction mixture was identical to that in Fig. 1 except the enzyme concentration, which is indicated in relative value. (a), 180-fold diluted enzyme solution was assayed; (b), 120-fold; (c), fold; (d) 30-fold; (e), control (without enzyme). Readings of the absorbance were carried out as described in text.

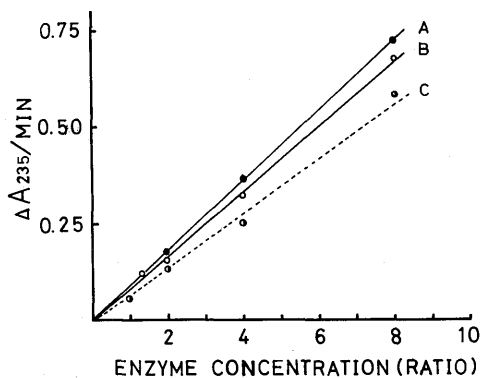


Fig. 3. Dependence of the initial reaction velocity on the substrate concentration.

The reaction mixture contained 2.0 ml of 0.1 (A), 0.05 (B) and 0.02 % (C) of alginic acid in 0.1 M Tris-HCl buffer (pH 8.3) and 0.2 ml of the enzyme solution in the different concentrations, which are indicated in relative value, as shown in figure. Ratio of the enzyme concentration was as follows. (1), 240-fold diluted enzyme solution was assayed; (2), 120-fold; (4), 60-fold; (8), 30-fold. Readings of the absorbance were carried out as described in text.

centrations. Effect of the substrate concentration on the initial reaction velocity is shown in Fig. 3. These facts indicate that the initial reaction velocity can be easily obtained from the straight line within a few minutes just after the initiation of the reaction, and is proportional over the wide range of the enzyme concentration in a fixed substrate concentration.

Alginate lyase activity has been determined so far by various methods. Those methods are complicated so as not to allow rapid determination. However, one of them is an ultraviolet absorption method, in which the absorbance at 235 nm is measured on the supernatant after removal of insoluble matters precipitated upon addition of perchloric acid to the reaction mixture.

The assay procedure investigated here is found to be very simple and rapid as described above, and it should be recommended in the measurement of the kinetic parameters of enzyme reaction, in which the initial reaction velocity is required more precisely.

REFERENCES

- 1) F.G Fisher and H. Dörfel: *Z. physiol. Chem., Hoppe-Seyler's* **301**, 224 (1955)
- 2) A. Haug and B. Larsen: *Acta Chem. Scand.*, **15**, 1395 (1961)
- 3) A. Haug and B. Larsen: *ibid.*, **16**, 1908 (1962)
- 4) I. Tsujino and T. Saito: *Nature*, **192**, 970 (1961)
- 5) I. Tsujino and T. Saito: *Agr. Biol. Chem.*, **26**, 115 (1962)
- 6) J. Preiss and G. Ashwell: *J. Biol. Chem.*, **237**, 309 (1962)
- 7) I. Tsujino and T. Saito: *Bull. Jap. Soc. Sci. Fish.* (in Japanese), **29**, 58 (1963)
- 8) Y. Ando and K. Inoue: *ibid.* (in Japanese), **31**, 552 (1965)
- 9) H. I. Nakada and P. C. Sweeny: *J. Biol. Chem.*, **242**, 845 (1967)
- 10) K. Nisizawa, S. Fujibayashi and K. Kashiwabara: *J. Biochem.*, **64**, 25 (1968)
- 11) H. I. Nakada, J. B. Wolfe, L. I. Hochstein and A. J. Andreoli: *Anal. Biochem.*, **2**, 168 (1960)