Spectrophotometric Determination of Cyclization Activity of \(\beta\)-Cyclodextrin-Forming Cyclomaltodextrin Glucanotransferase

Takahiro KANEKO, Takashi KATO, Nobuyuki NAKAMURA* and Koki HORIKOSHI

Laboratory of Applied Bacteriology, The Institute of Physical and Chemical Research (RIKEN)
(2-1, Hirosawa, Wako, Saitama 351, Japan)

(Received November 29, 1986)

A quick and reliable assay was developed to measure the cyclization activity of \(\beta\)-cyclodextrin-forming cyclomaltodextrin glucanotransferase. Beta-cyclodextrin formed in the reaction was measured directly in situ through as a complex with phenolphthalein. Using this assay, it was shown that the crude enzyme from an alkalophilic Bacillus sp. (ATCC 21783) showed cyclization activity with three distinct pH optima, at pH 4.5, 6.0 and 8.5. Soluble starch and maltohexaose were better substrates than other smaller maltooligosaccharides.

Cyclomaltodextrin glucanotransferase (EC 2.4.1.19, CGTase) catalyzes the formation of cyclodextrins (CDs), which are non-reducing cyclic maltooligosaccharides composed of \(\alpha\)-1,4-linked D-glucose molecules.\(^{1-5}\) The existence of branched CDs is also well known.\(^ {5,7}\) Industrially, CGTase is used to produce CDs,\(^ {5,9}\) sweetners\(^ {10}\) and maltooligosaccharides.\(^ {11}\) There have been many reports on the production of bacterial CGTases, which are divided into two types, one which forms \(\alpha\)-CD from starch and one which forms \(\beta\)-CD from starch mainly.\(^ {12-21}\) The CGTase from \(B.\) stearothermophilus seems to be intermediate between these two types.\(^ {18,22}\) Although the enzyme has a similar function to \(\alpha\)-amylase, i.e., it can liquefy starch pastes, which results in a reduction in the colour intensity of the starch-iodine complex, it is also able to catalyze the synthesis of CDs from starch pastes or related material (cyclization), the reversible ring opening and closure of CDs in the presence of certain co-substrates such as glucose, maltose, sucrose and some saccharides (coupling reaction), and the formation of higher and lower maltooligosaccharides at rather high substrate concentrations (homologizing or disproportionation reaction).\(^ {13}\) Studies on the properties of CGTases have usually concentrated upon the dextrinogenic activity or transglycosylation activity of the enzymes.\(^ {9,12-15,20-24}\)

Little information is available on the cyclization activity, particularly in the case of the \(\beta\)-CD-forming enzymes.\(^ {19}\) Assays for \(\alpha\)-CD-forming activity have been reported but there is no simple procedure for measuring \(\beta\)-CD-forming activity.\(^ {25,26}\)

Recently, the stability constants of some CD-inclusion complexes were determined by utilizing the competition of guest compounds for phenolphthalein or methylorange.\(^ {27}\) By modifying this assay, we developed a simple, quick and specific assay for the cyclization activity of \(\beta\)-CD-forming CGTases.

The reduction in the colour intensity of phenolphthalein after complexation with \(\alpha\), \(\beta\)-
or \(\gamma\)-CD is shown in Fig. 1. The colour intensities of inclusion complexes of \(\beta\)- and \(\gamma\)-CDs decreased in a linear manner with increasing amounts of the CDs up to 0.5 mg of \(\beta\)-CD and 2.0 mg of \(\gamma\)-CD, respectively. However, no absorbance change was observed in the case of \(\alpha\)-CD. Soluble starch and maltooligosaccharides did not affect the colour intensity of phenolphthalein (data not shown).

Figure 2 shows the changes in cyclization activity of the crude \(\beta\)-CD-forming CGTase from an alkalophilic Bacillus sp. (ATCC 21783), which was supplied by Nihon Shokuhin Kako Co., when soluble starch (Kanto Chemical Co.), which was washed twice with deionized water before use, and a series of maltooligosaccharides (Nihon Shokuhin Kako Co.) were used as substrates.

The cyclization activity was determined as follows: a reaction mixture containing 40 mg of each substrate in 1.0 ml of 0.1 M Na phosphate buffer (pH 6.0) and 0.1 ml of CGTase solution suitably diluted with deionized water was incubated at 60°C for 20 min. The reaction was stopped by adding NaOH and phenolphthalein, as shown in Fig. 1, and then the colour intensity was measured as described above. Blanks lacking the enzyme were run with each batch of assays. As a standard, the soluble starch and enzyme were replaced by 0.5 mg of \(\beta\)-CD and 0.1 ml of water, respectively. One unit of enzyme activity was defined as the amount of enzyme that formed 1 mg of \(\beta\)-CD per min under the conditions described above.
Dextrinizing power was assayed at 60°C with 0.2% (w/v) potato amylase (Wako Pure Chemical Ind. Co.) solution (pH 6.0) as a substrate by the method of Fuwa.\textsuperscript{28} One unit of dextrinizing power was defined as the amount of enzyme that produced a 10% reduction in the intensity of the blue colour of the amylose-iodine complex. Using the reduction in colour intensity of phenolphthalein as a measure of cyclization, for all the substrates tested, the cyclization activity was found to vary in a linear manner with dextrinizing power from 1.5 to 3.7 U/ml. The enzyme activity increased with an increase in molecular weight of the maltooligosaccharide substrates. Soluble starch was the most effective substrate tested. The pH profile for the cyclization activity of the crude β-CD-forming CGTase of an alkalophilic Bacillus sp. is shown in Fig. 3. It can be seen that at 60°C cyclization is most active around pH 5.5, 6.0 and 8.5. When the pH profile for cyclization activity was obtained at 40°C, the results resembled those in the case of 60°C, with the lowest pH optimum having shifted to around pH 4.5.

The complexity of the pH profile suggests that there may be three extracellular enzymes showing cyclization activity in the culture supernatant, each one active in a different pH region.

Therefore, measurement of the reduction in colour intensity of phenolphthalein following its incorporation into the cavity of β-CD is a reliable and quick method for determining the cyclization activity of β-CD-forming CGTases. This assay procedure should prove useful for characterizing this property of certain CGTases and for studying the secretion mechanisms involved.

We are grateful to Dr. K. N. Joblin of our laboratory for the editorial assistance with this report and to Miss H. Nagayama for her technical assistance in this work.

REFERENCES

比色法によるβ-CD 生成型 CGTase の
CD 合成活性の測定

金子隆宏*, 加藤 卓*, 中村信之*,**,
摂越恵毅*

* 理化学研究所（351 埼玉県和光市広沢 2-1）
** 現在、日本食品工業研究所
（417 静岡県富士市田島 30）

β-CD 生成型 CGTase の CD 合成活性を フェノールフレインを用いて測定した。活性測定の基質には可溶性澱粉がもっとも適していた。本方法を用いて好アルカリ性バチルス（ATCC 21783）の培養ろ液から得た粗 CGTase の三つの pH 領域に β-CD 生成反応の至適 pH があり、粗酵素中には性質を異にする三つの CGTase
が含まれていることを示していた。