●TOYOBO ENZYMES●

(Diagnostic Reagent Grade)

GLUCOAMYLASE

from Rhizopus sp.

1,4-α-D-Glucan glucohydrolase (EC 3.2.1.3)

Starch + n H_2O ⟷ n β-D-Glucose

PREPARATION and SPECIFICATION

Appearance	: White amorphous powder (salt-free), lyophilized
Activity	: Grade Ⅰ 30U/mg-solid or more

PROPERTIES

Stability	: Stable at −20℃ for at least one year (Fig.1)
Molecular weight	: approx. 70,000 [1]
Michaelis constants [1]	: 11±1.1×10^{-4}M (Maltose), 3.6±0.51×10^{-4}M (Maltotriose), 2.5±0.33×10^{-4}M (Maltotetraose), 1.6±0.02×10^{-4}M (Maltopentaose)
Structure	: Glycoprotein [E^{280nm}_{1cm} (1%)=14.5]
Optimum pH	: 4.5−5.0 (Fig.3)
Optimum temperature	: 60℃ (Fig.4)
pH Stability	: pH 4.0−8.5 (25℃, 20hr) (Fig.5)
Thermal stability	: below 45℃ (pH 5.5, 10min) (Fig.6)
Substrate specificity [1,2]	: This enzyme completely hydrolyzes soluble starch, amylopectin, glycogen, α-or β-limit dextrin, amylose, maltooligosaccharides and panose.

APPLICATIONS

This enzyme is useful for structural investigation of carbohydrates and for enzymatic determination of α-amylase when coupled with the related enzymes in clinical analysis.

ASSAY

Principle:

Starch + n H_2O $\xrightarrow{\text{glucoamylase}}$ n Glucose + Dextrin

The formation of glucose is measured as reducing sugar by the modified Fehling-Lehmann-Schoorl method.

Unit definition:

One unit causes the formation of ten milligrams of glucose in 30 minutes under the conditions described below.

Method:

Reagents

A. Starch solution : 1.0% [Suspend 1.0g of soluble starch (Merck) in 90ml of H_2O, dissolve by boiling for 3min and cool down to room temperature. Add 5.0ml of 1.0M acetate buffer, pH 4.5 and fill up to 100ml with H_2O.] (Should be prepared fresh)

B. Alkaline solution : 100g NaOH, 365g Rochelle salt · $4H_2O$/1,000ml of H_2O

C. $CuSO_4$ Solution : 7.0% (70g $CuSO_4 \cdot 5H_2O$/1,000ml of H_2O)

D. KI solution : 30% (300g KI/1,000ml of H_2O)(Store in a brownish bottle)

E. H_2SO_4 Solution : 25%

F. $Na_2S_2O_3$ Solution : 50mM (49.638g $Na_2S_2O_3 \cdot 5H_2O$, 4.0g Na_2CO_3 (stabilizer)/4,000ml of H_2O)(Store in a brownish bottle and keep for 3～4 days before use)

G. Enzyme diluent : 10mM acetate buffer, pH 4.5

Procedure

1. Pipette 4.0ml of substrate solution (A) into a test tube (32ϕ × 200mm) and equilibrate 40℃ for about 5minutes.
2. Add 1.0ml of the enzyme solution* and mix.
3. After exactly 15 minutes at 40℃, add 2.0ml of alkaline solution (B) stop the reaction.
   At the same time, prepare the blank by first mixing the substrate solution with 2.0ml of alkaline solution after 15min-incubation at 40℃, followed by addition of the enzyme solution.
4. Add 2.0ml of $CuSO_4$ solution (C) and, after covering the test tube with a marble (40mmϕ) to prevent evaporation, place the test tube in a boiling water bath.
5. After 20 minutes, remove the test tube from a boiling water bath and cool down to room temperature under running water.
6. Add 2.0ml each of KI solution (D) and H_2SO_4 solution (E) in this order.
7. Shake the test tube and determine the amount of residual Cu^{++} by titration with $Na_2S_2O_3$ solution (F).
8. Record the titers (ml) of the test (Δt) and the blank (Δb), and calculate the titration difference in ml (Δ sample: $\Delta b - \Delta t$).

Concentration in assay mixture	
Acetate buffer	42 mM
Starch	0.8 %

* Dissolve the enzyme preparation in ice-cold distilled water and dilute to 0.4－1.5U/ml with enzyme diluent (G), immediately before assay.

Calculation

Activity can be calculated by using the following formula :

$$\text{Volume activity (U/ml)} = \frac{\Delta\text{sample} \times 30\text{min} \times \text{df}}{\Delta\text{glucose} \times 15\text{min}} = \frac{\Delta\text{sample}}{\Delta\text{glucose}} \times 2.0 \times \text{df}$$

Weight activity (U/mg) = (U/ml) × 1/C

Δglucose: Titration difference (ml) for ten miligrams of glucose (Determine the titration difference by using glucose standard solution (5.0mg/ml) instead of the enzyme solution under the above assay conditions.)

df : Dilution factor

C : Enzyme concentration in dissolution (c mg/ml)

REFERENCES

1) K.Hiromi, Y. Nitta, C.Numata and S.Ono; *Biochim.Biophys.Acta, 302,* 362 (1973).

2) J.Fukumoto; *Protein, Nucleic Acid and Enzyme, 4,* 3 (1959).

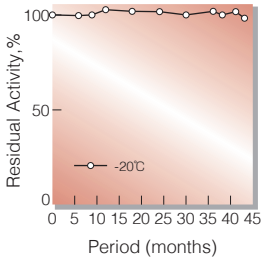

Fig.1. Stability (Powder form)
〔kept under dry conditions〕

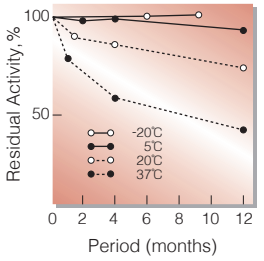

Fig.2. Stability (Powder form)
〔kept under dry conditions〕

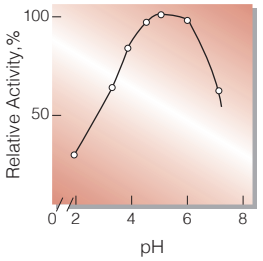

Fig.3. pH-Activity
〔40℃,15min-reaction in 50mM buffer solution: pH2.0,sodium acetate-HCl; pH3.0-6.0,acetate;pH6.0-7.0, phosphate〕

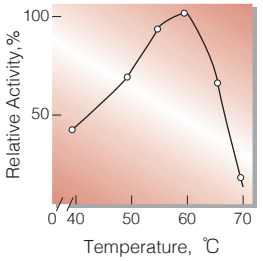

Fig.4.Temperature activity
〔15min-reaction in 50mM acetate buffer,pH4.5〕

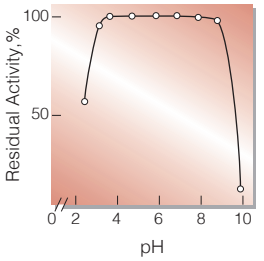

Fig.5.pH-Stability
〔25℃,20hr-treatment with 50mM buffer solution: pH3.0-6.0 acetate; pH6.0-9.0,phosphate;pH9.0-10.0, borate〕

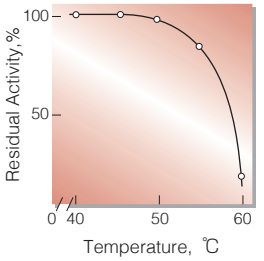

Fig.6.Thermal stability
〔10min-treatment with 50mM acetate buffer,pH5.5〕

活性測定法（Japanese）

1.原理

Starch ＋ n H_2O $\xrightarrow{\text{glucoamylase}}$ n Glucose＋Dextrin

グルコース(還元糖)の生成量をフェーリング・レーマン・ショール変法で測定する。

2.定義

下記反応条件下で30分間に10㎎のグルコースを生成する酵素量を1単位 (U)とする。

3.試薬

A. 1.0％可溶性澱粉溶液〔1.0gの可溶性澱粉(Merck製)を90㎖の蒸留水に懸濁後，約3分間煮沸溶解する。室温迄冷却後，1.0M酢酸緩衝液，pH4.5を5.0㎖添加し，最終液量を蒸留水で100㎖とする〕(用時調製)

B. ロッシェル塩アルカリ溶液(100gのNaOH及び365gの酒石酸カリウム・ナトリウム塩・4H_2Oを蒸留水に溶解し，1000㎖とする)

C. 7.0%硫酸銅溶液(70gの$CuSO_4$・5H_2Oを蒸留水に溶解し，1,000㎖とする)

D. 30%ヨードカリ溶液(300gのKIを蒸留水に溶解し，1,000㎖とする)(褐色瓶中で保存)

E. 25%硫酸溶液

F. 50mMチオ硫酸ナトリウム溶液〔49.638gの$Na_2S_2O_3$・5H_2O及び4.0gのNa_2CO_3(安定化剤)を蒸留水に溶解し，4,000㎖とする〕(褐色瓶中で保存し，調製後3～4日放置して使用する)

酵素溶液：酵素標品を予め氷冷した蒸留水で溶解し，分析直前に10mM酢酸緩衝液 pH4.5で0.4～1.5 U/㎖に希釈する。

4.手順

①試験管(32φ×200mm)に基質溶液 (A)4.0㎖を採り，40℃で約5分間予備加温する。
②酵素溶液を1.0㎖を加え，反応を開始する。
③40℃で正確に15分間反応させた後，ロッシェル塩アルカリ溶液 (B) 2.0㎖加えて反応を停止させる。
④硫酸銅溶液 (C)を2.0㎖加え,試験管上に40mmφのガラス玉をのせ(蒸発防止)沸騰浴中で20分間煮沸する。
⑤流水中で室温迄冷却する。
⑥ヨードカリ溶液 (D) 2.0㎖及び硫酸溶液 (E) 2.0㎖をこの順序に加える。
⑦よく混和した後,チオ硫酸ナトリウム溶液 (F)で滴定する。→(反応滴定値)
⑧盲検は基質溶液 (A) 4.0㎖を40℃で15分間放置後，ロッシェル塩アルカリ溶液(B) 2.0㎖を加えて混和し,次いで酵素溶液1.0㎖を加えて調製する。以下手順④～⑦を操作して滴定値を求める。→(盲検滴定値)

5.計算式

$$U/㎖ = \frac{(\text{盲検滴定値}-\text{反応滴定値})\times 30(分)\times \text{希釈倍率}}{\text{標準滴定値}\times 15(分)}$$

$$= \frac{(\text{盲検滴定値}-\text{反応滴定値})}{\text{標準滴定値}} \times 2.0 \times \text{希釈倍率}$$

U/mg ＝U/㎖×1/C

標準滴定値：酵素溶液の代りにグルコース標準溶液(5.0㎎/㎖)を用いて上記手順に従って操作し,グルコース10㎎に相当する滴定値を算出する(内部標準)

C：溶解時の酵素濃度(c ㎎/㎖)