17 th INTERNATIONAL BIOLOGY OLYMPIAD
9-16 JULY 2006
Río Cuarto – República Argentina

PRACTICAL TEST

3

Biochemistry

生物化學

Student code: 學生代碼	

17 th INTERNATIONAL BIOLOGY OLYMPIAD
9-16 JULY 2006
Río Cuarto – República Argentina

General remarks about the practical tests

DEAR PRATICIPANS **參賽者**

The practical test are organized in four different laboratories. 實作在 4 間不同實驗室進行

Nº 1- Plant Anatomy, Systematics and Physiology 植物解剖學，分類學和生理學

Nº 2- Animal Anatomy, Systematics and Ecology 動物解剖學，生態學和分類學

Nº 3- Biocheminstry 生物化學

Nº 4- Microbiology 微生物學

- You have **1 hour** in laboratories Nº 1 and Nº 2. 實驗 1 及 2 為 1 個小時
- You have **1 hour 30 minutes** in laboratories Nº 3 and Nº 4. 實驗 3 及 4 為 1.5 個小時
- You can score maximum **40 points** in each laboratory, which means a total of **160 points** for the practical test. 每個實驗你最多可得 40 分，實作試驗共 160 分

Good luck !!!!!!!

Practical test Nº 3: Biochemistry

Enzymatic determination of glucose

利用酵素反應測定葡萄糖濃度

TASK 1: You have to perform a calibration curve using a standard of glucose, with known concentration. Then, plot the results as absorbance versus glucose concentration (15 points)

實作一：利用已知濃度的葡萄糖溶液，繪製濃度曲線圖。並將測量結果按吸光值對應葡萄糖濃度的方式標示在圖中。**(15** 分**)**

Important: Raise the red card to call the lab assistant when you are ready to use the spectrophotometer.

重要事項：準備使用光電比色計時，請舉起紅色卡片通知實驗室助理人員。

Introduction: 內容介紹

Glucose oxidase (GOD) catalyzes the oxidation of (beta)-D-glucose to D-gluconic acid and hydrogen peroxide. It is highly specific for (beta)-D-glucose and does not act on (alpha)-D-glucose. The horseradish peroxidase (POD) breaks down hydrogen peroxide into water and oxygen, using the dye (4-aminophenazone) as an electron donor. At the same time, the dye is converted to its oxidized form, which is a colored compound. Since the amount of hydrogen peroxide produced indicates how much reaction has taken place, the formation of the red color can be used to follow the course of the reaction.

葡萄糖氧化酶(GOD)可催化β-D-葡萄糖，轉換成 D 型葡萄糖酸及過氧化氫的氧化作用，GOD 對β-D-葡萄糖具有高度的專一性，而 horseradish 過氧化酶(POD)可利用染色劑所提供

的電子，將過氧化氫分解成水及氧，此時染色劑會因氧化而呈現紅色。由於過氧化氫的生成量與反應強度呈正比，故可藉由紅色的深淺估算反應的大小。

Its major use is in the determination of free glucose in body fluids. Although specific for (beta)-D-glucose, glucose oxidase can be used to measure total glucose, because as a result of the consumption of (beta)-glucose, (alpha)-glucose from the equilibrium is converted to the (beta)-form by mutarotation.

此方法可用以計算體液中葡萄糖的濃度，儘管 GOD 對β-D-葡萄糖具備高度的專一性，GOD 仍可用於估算體液中全部葡萄糖的濃度，因為當身體使用β-D-葡萄糖後，α-D-葡萄糖會藉由變旋作用轉換成β-D-葡萄糖，以維持β-型及α型葡萄糖間的濃度平衡。

PRINCIPLE 實驗原理

The reaction system is as follows: 其反應式如下圖

GOD (葡萄糖氧化酶)

Glucose (葡萄糖)+ O_2 + H_2O----------> Gluconic Acid (葡萄糖酸)+ H_2O_2

POD

2 H_2O_2 + 4-AP + 4-Hydroxybenzoate---------- > red quinoneimine

Glucose oxidase reagent: solution containing glucose oxidase, peroxidase, 4-aminophenazone (4-AP), and phosphate buffer pH 7.0 containing hydroxybenzoate.

葡萄糖氧化酶反應試劑成份：包括 GOD、過氧化氫酶、4-AP (染料)等。

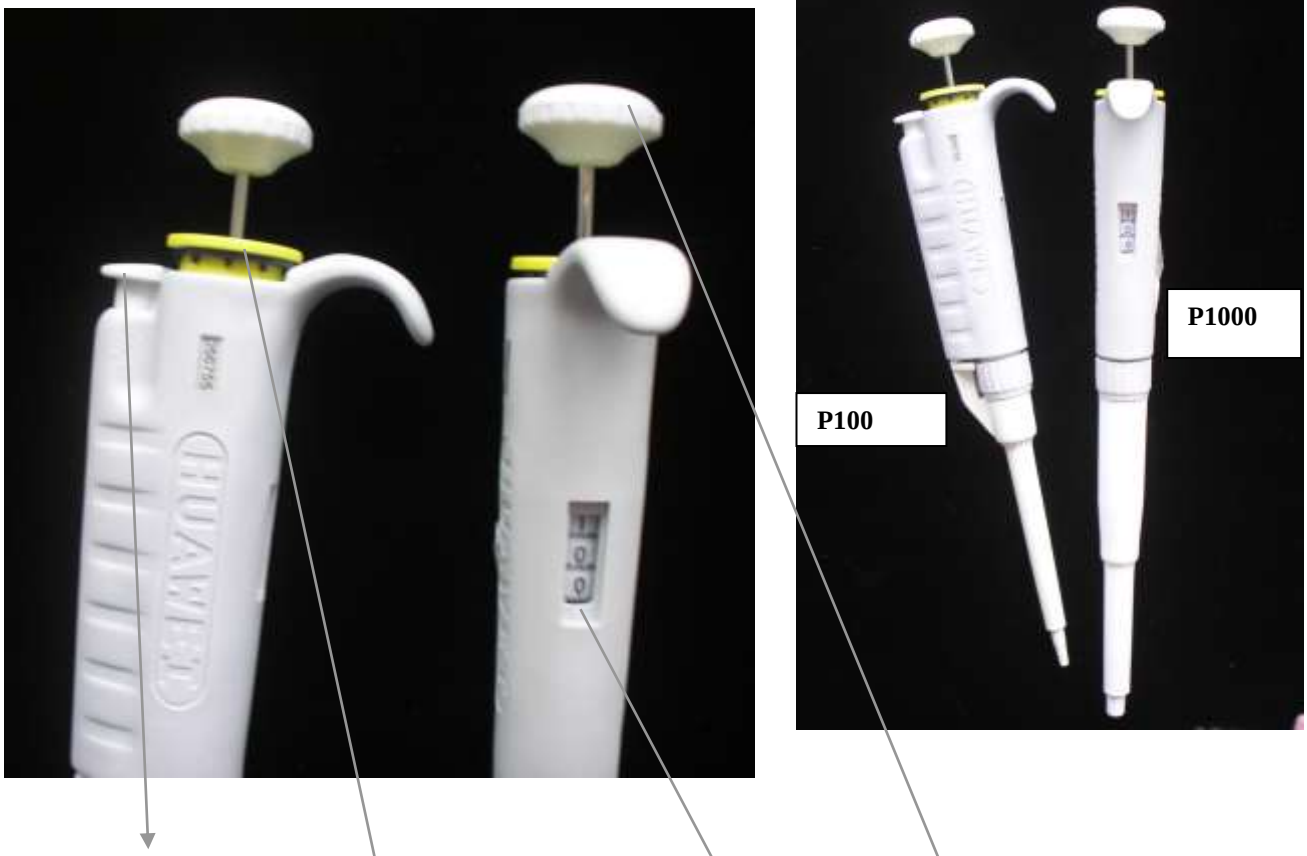
Reagents: 本實驗中的反應試劑

1. glucose oxidase reagent (ready to use). 葡萄糖氧化酶反應試劑 (已配妥可馬上使用)
2. glucose solution (unknown concentration). 葡萄糖溶液 (其濃度未知)
3. glucose solution 5 mg. ml^{-1}. 葡萄糖溶液 (濃度為 5 mg/mL)
4. distilled water. 蒸餾水

Equipment 本實驗中各項設備

1. Lab gloves (1pair). 實驗手套 (一對)
2. Marker pen (1). 油性筆 (一支)
3. 1.5 ml microtubes (18). 1.5 ml 離心管 (18 個)
4. Pipettes (2). 微量滴管 (兩支)
5. Incubator at 37°C . 已設定於 37°C 的水浴器
6. Spectrophotometer (you will use it with lab assistants).光電比色計 (需在助理協助下使用)
7. Spectrophotometric cuvettes (8). 比色管 (8 個)
8. Paper towels (3) 紙巾 (3 包)
9. 1000 µl tips (30) 1000 µl 滴管尖 (30 個)
10. 200 µl tips (30) 200 µl 滴管尖 (30 個)

Instruments: 實驗儀器



Adjustment method 調整方法

You have to pull up the Adjustable Wheel, then you can revolve the adjustable wheel or knob. Adjust the required volume and push down the Adjustable Wheel.

Remember that minimal and maximal volumes for P100 are 10 µl and 100 µl respectively.

For P1000 minimal volume is 100 µl and maximal volume is 1000 µl.

先拉起調整輪，再轉動調整輪，調整至所需容量後，再壓下調整輪。

注意：P100 微量滴管的最小及最大容量分別為 10 µl 及 100 µl。而 P1000 微量滴管的最小及最大容量分別為 100 µl 及 1000 µl。

Usage method: 使用方法

Please secure the suction tip, after that slightly push down the pushing knob to first stop, hold and immerse the tip into solution vertically. The immersed depth of the tip is 2-4 mm, then release the pushing knob slowly and make it return to the original position. Take off the pipette from the liquid and place the suction tip of the pipette into a special container receiving the dispensed liquid. The tip must be close to the inner wall of the container. Depress the pushing knob to the first stop and further more to discharge the solution completely from the tip. After that, you can take away the pipette and release the button. Eject the used tip to the trash recipient by pressing the Tip ejecting knob.

裝上滴管尖，輕輕壓下按鈕直到首個停止位，將滴管尖以垂直方式置入溶液中，約在液面下 2-3mm 處，放開按鈕使其慢慢回復原位並抽起的液體，再將管尖置入容器的內壁，重新壓下按鈕排放液體，要壓至首個停止位更下方的位置，確保所有液體均被排出，壓下排放滴管尖按鈕，可將使用過的管尖移除。

EXPERIMENTAL PROCEDURE 實驗流程

1) Label five 1.5-ml microtubes 1/2 through 1/32 with a marker pen. Using the glucose standard solution (5 mg. ml $^{-1}$) perform the following serial dilutions (in distilled water) in a final volume of 100 µl: 1/2, 1/4, 1/8, 1/16, and 1/32.

利用油性筆於五個 1.5 ml 微量離心管上，標示 1/2、1/4、1/8、1/16 及 1/32 等五個稀釋比例數值，將濃度為(5 mg/ml)的葡萄糖溶液，按照名離心管上所標示的稀釋比例，利用蒸餾水進行序列稀釋，各試管內稀釋後的葡萄糖溶液，其最終體積設定為 100 µl。

2) Mix well and perform (in a new 1.5-ml microtubes set) the enzymatic determination of glucose for each dilution according to the following squeme.

混合均勻後，將稀釋的葡萄糖溶液，依下表所示體積，分別加到新的 1.5 ml 離心管中。

	1/2	1/4	1/8	1/16	1/32	Blank
Sample volume 樣本的體積	10µl	10µl	10µl	10µl	10µl	0
Water volume 蒸餾水的體積	0	0	0	0	0	10 µl
Glucose oxidase reagent volume 葡萄糖氧化酶 反應試劑的體積	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml

3) Mix well and incubate microtubes at 37°C for 5 min.

混合均勻後將各離心管放入 37°C 水浴器中反應五分鐘。

4) Put the content of each microtube in a spectrophotometric cuvette.

將各離心管的內容物，分別移入比色管中。

5) Read absorbance in a spectrophotometer at 505 nm. You have to use the blank for calibration. (**When you are ready, to read in the spectrophotometer, please call the lab assistant)**

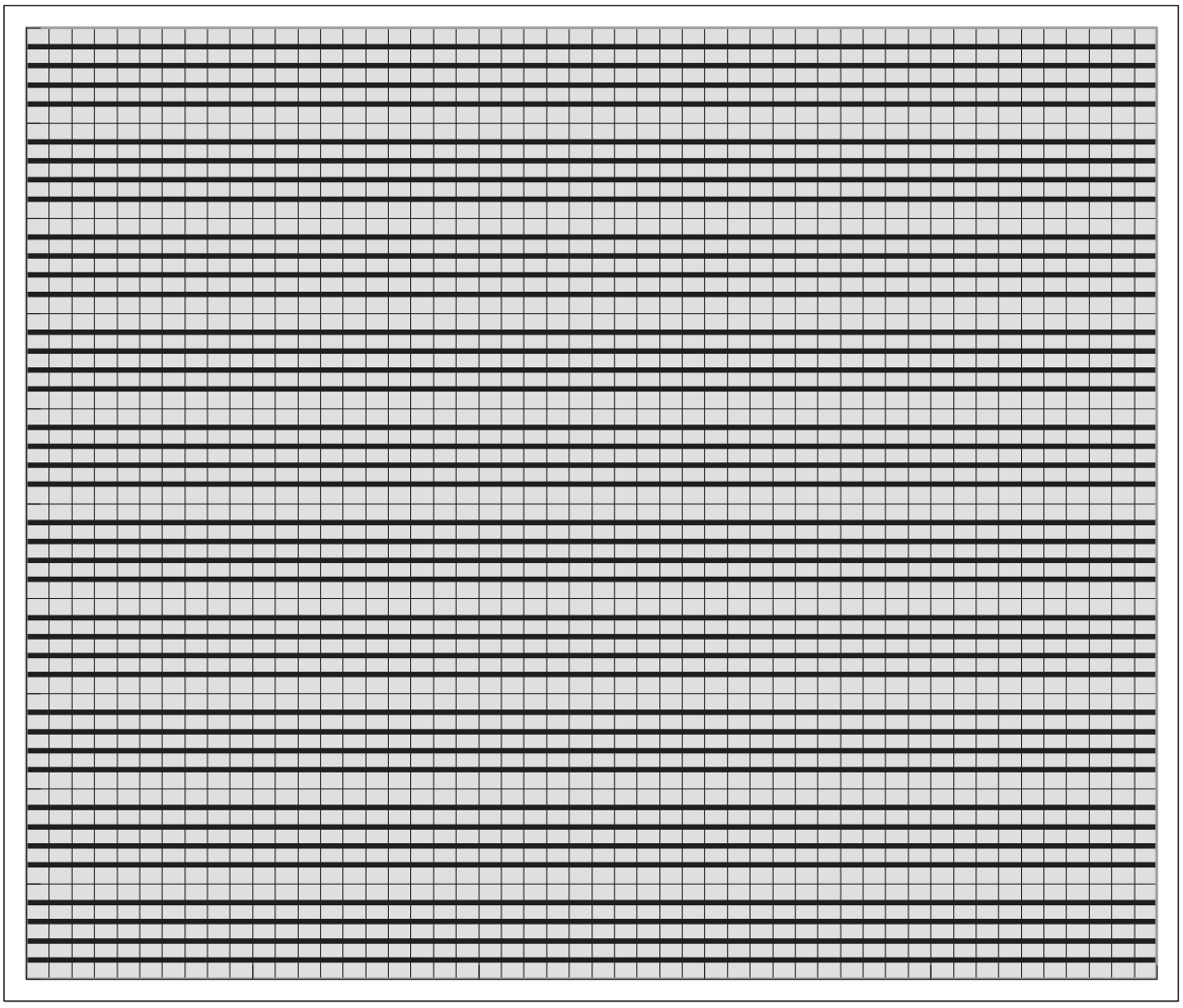
必需使用一個空白的樣本進行較正，讀取各比色管於 505 nm 下的吸光值。

(當你/妳準備要使用光電比色計時，請舉起紅色卡通知實驗室助理人員)

6) Plot the absorbance versus the amount of glucose (in µg) on the plotting paper below.

將吸光值與對應的葡萄糖水濃度(請換算為µg)畫在下方的記錄紙中。

	Dilutions (稀釋比例)				
	1/2	1/4	1/8	1/16	1/32
glucose (µg in the reaction mix) 反應物中葡萄糖濃度(µg)					
Absorbance at 505 nm 於波長 505nm 時的吸光值					



TASK 2: Determination of the glucose concentration in a sample employing the standard curve obtained before. (10 points)

實作 2：利用先前畫出的標準曲線，求出樣本中葡萄糖的濃度。(10 分)

1) Perform the glucose oxidase reaction, to the glucose sample (unknown concentration), according to the follow scheme.

按照下表所示，加入未知濃度的葡萄糖樣本，針對新的樣本，使用相同的葡萄糖氧化酶反應。

	Sample	Blank
Sample volume 樣本的體積	10µl	0
Water volume 蒸餾水的體積	0	10 µl
Glucose oxidase reagent volume 葡萄糖氧化酶 反應試劑的體積	1 ml	1 ml

2) Mix well and incubate microtubes at 37°C for 5 min.

混合均勻後將各離心管放入 37°C 水浴器中反應五分鐘。

3) Put the content of each microtube in a spectrophotometric cuvette

將各離心管的內容物，分別移入比色管中

4) Read absorbance in the spectrophotometer at 505 nm. You have to use the blank for calibration. **(When you are ready to read in the spectrophotometer please call the lab assistant)**

必需使用一個空白的樣本進行校正，讀取各比色管於 505 nm 下的吸光值。

(當你/妳準備要使用光電比色計時，請舉起紅色卡通知實驗室助理人員)

5) Using the calibration curve, calculate the glucosa concentration of the sample in µg. ml^{-1}.
利用先前劃出的標準曲線，計算出樣本中葡萄糖的濃度 (必需以µg/ml 表示求得之葡萄糖濃度及吸光值的關係)。

Absorbance of the sample 樣本的吸光值	
Concentration of the sample (in µg . ml^{-1}) 樣本中的葡萄糖濃度 (µg/ml)	

Question 1 已刪除，不要做。

QUESTION 1: As many glucose assays measure the peroxide produced by the glucose oxidase reaction, it is important that the enzyme used for these assays presents: **(1.5 points) Deleted** 已刪除，不要做。

問題 1：藉由葡萄糖氧化酶反應所產生的過氧化氫測量葡萄糖的濃度，用以反應的酶需具備下列何種特性？ Deleted 已刪除，不要做。

A) a low catalase content. 一個較低的催化酶 content 已刪除，不要做。

B) a high catalase content. 一個較高的催化酶 content 已刪除，不要做。

C) a low peroxidase content. 一個較低的過氧化酶 content 已刪除，不要做。

D) a high peroxidase content. 一個較高的過氧化酶 content 已刪除，不要做。

WRITE DOWN THE LETTER CORRESPONDING TO CORRECT ANSWER

將代表正確答案的英文字母填入答案卷的欄位中已刪除，不要做。

Answer:.....Deleted..... 已刪除，不要做。.....

QUESTION 2: Glucose oxidase reagent may contain catalase. If such a condition is not taken into account the obtained results will give **(1.5 points)**:

問題二： 葡萄糖氧化酶反應溶液中，可能含有觸酶(氧化去氫酶)，如不考慮此項條件，則對實驗結果的判讀，可能造成下列何種影響(1.5 分)

A) underestimation of the glucose in the assay. 低估葡萄糖的濃度

B) overestimation of the glucose in the assay. 高估葡萄糖的濃度

C) not effect in the assay. 無任何影響

WRITE DOWN THE LETTER CORRESPONDING TO CORRECT ANSWER

將代表正確答案的英文字母填入答案卷的欄位中

Answer:.....

QUESTION 3: The most favorable pH value (the point at which the enzyme is most active) is known as the optimum pH. Extremely high or low pH values usually result in a complete loss of enzyme activity due to **(1 point)**:

問題 3：最佳反應酸鹼值，其定義為可使酵素反應活性最大的 pH 值。極高或極低的 pH 值常使酵素完全失去活性，其原因為何？**(1 分)**

A) The breakdown of the secondary structure of the protein. 破壞了蛋白質的二級構造。

B) The breakdown of the tertiary structure of the protein. 破壞了蛋白質的三級構造。

C) The breakdown of the primary structure of the protein. 破壞了蛋白質的一級構造。

SELECT ONLY ONE CORRECT ANSWER MARK IT WITH A CROSS.

請在代表正確答案的空格內打”X”。

☐ A. ☐ B. ☐ C. ☐ A, B

☐ A, C ☐ B, C. ☐ A, B, C.

QUESTION 4: Glucose oxidase from the fungus *Aspergillus niger* was overexpressed in yeast. The glucose oxidase was purified and glycosylation pattern was analyzed by treatment with endoglycosidase H and α-mannosidase. After treatment, an aliquot was used for SDS-PAGE (electrophoresis in polyacrylamide gels containing sodium dodecyl sulphate) in reducing conditions. The remaining enzyme was employed for determination of the K_M (Michaelis-Menten constant) with glucose as the substrate. Michaelis-Menten constant ($K_{M)}$ is the concentration in moles/litre of a substrate at half the maximum velocity of an enzymatic reaction. **(7 points)**

問題四：利用酵母菌大量表現黑麴菌的葡萄糖氧化酶，將其葡萄糖氧化酶純化後，利用內糖苷酶及α-甘露糖酶處理後，分析其糖基型式的改變對其酵素活性的影響。經酵素處理後的葡萄糖氧化酶，先用 SDS-PAGE 進行分離，再利用葡萄糖當作反應受質，分析其酵素活性，並計算其 K_M 值。K_M 值代表某酵素達到其最大反應率一半時所需的受質濃度(單位為 moles/litre)(7 分)

The values of the K_M for each glycoforms are shown below the figure 1.

具有不同型式糖基的葡萄糖氧化酶的 K_M 值列於下圖。

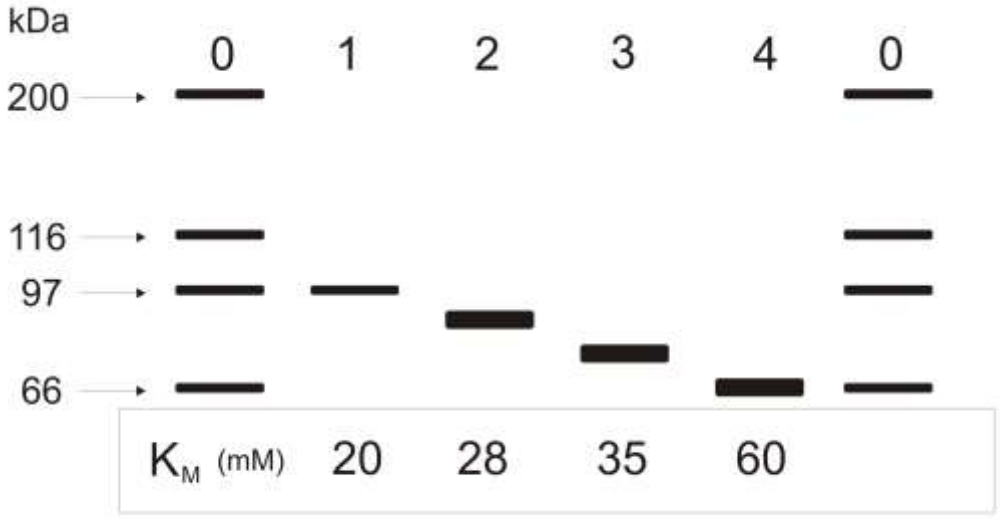


Figure 1: Analysis of the deglycosylation of Glucose

Oxidase by 7.5% acrylamide SDS-PAGE gel electrophoresis. Lane 0 is the molecular mass standard. Lane 1 untreated enzyme. Lane 2 endoglycosidase H treated enzyme. Lane 3 α-mannosidase treated enzyme. Lane 4 endoglycosidase H and α-mannosidase treated enzyme (fully deglycosylated enzyme).

圖 1：利用 7.5%的 SDS-PAGE 分析糖基型式改變的葡萄糖氧化酶，第一行為分子量標準液，第二行為未經酵素處理的葡萄糖氧化酶，第三行為經α-甘露糖酶處理後的葡萄糖氧化酶，第三行為經內糖苷酶及α-甘露糖酶處理後(完全去除糖基)的葡萄糖氧化酶。

Which of the following is/are correct conclusion(s) from these results obtained in the determination of KM for each glycoform. (figure 1)

根據圖 1 的結果，下列何者(或哪些)為正確的結論？

A) Glucose oxidase is a homodimer with a molecular mass of 96 kDa.

葡萄糖氧化酶為一同型二聚體，其分子量為 96 kDa.。

B) The deglycosylated form has a molecular mass of approximately 68 kDa.

去除糖基的葡萄糖氧化酶，其分子量約為 68 kDa.。

C) Glucose oxidase is glycosylated since the treatment with endoglycosidase H and α-mannosidase results in a form with lower molecular mass.

經內糖苷酶及α-甘露糖酶處理後的葡萄糖氧化酶，其分子量會減少，故可推論葡萄糖氧化酶是一個具有糖基的蛋白質

D) The polysaccharide moiety of glucose oxidase contains *N*-acetylglucosamine and mannose.

葡萄糖氧化酶的多醣構造中，含有 N-乙醯胺基葡萄糖及甘露糖。

MARK THE CORRECT ANSWER/ ANSWERS.

請在正確答案的空格內打乂。

□ A □ B □ C □ D

From the results obtained in the determination of K_M for each glycoform, the following conclusion could be made. Which of the following statements is/are correct?

根據測定改變糖基型式的葡萄糖氧化酶之 K_M 值的結果，下列何者(哪些)是正確的敘述？

A) The affinity of the fully glycosylated enzyme for glucose is higher than the affinity of the deglycosylated enzyme

糖基化葡萄糖氧化酵素，對葡萄糖的親和力較高。

B) The glucose oxidase activity is completely abolished in the deglycosylated form

去糖基化後的葡萄糖氧化酵素，完全失去其活性。

C) The lack of the sugar moiety could cause changes in the structure of the active site of the enzyme resulting in the observed modifications of the *K*Ms.

去除糖基會使葡萄糖氧化酵素的活化位構造改變，因而產生所觀察到的 K_M 值變化。

MARK THE CORRECT ANSWER/ ANSWERS.

請在正確答案的空格內打乂。

□ A □ B □ C

QUESTION 5: The purified enzyme was analyzed by SDS-PAGE in reducing (DDT+) as well as in non-reducing (DDT-) conditions. The obtained results are shown in figure 2 **(4 points)**

問題 **5**：純化後的葡萄糖氧化酵素，分別在還原劑存在**(DTT+)**及不存在**(DTT-)**的條件下，利用 **SDS-PAGE** 電泳法進行分離，圖 **2** 為實驗所得的結果。**(4** 分**)**

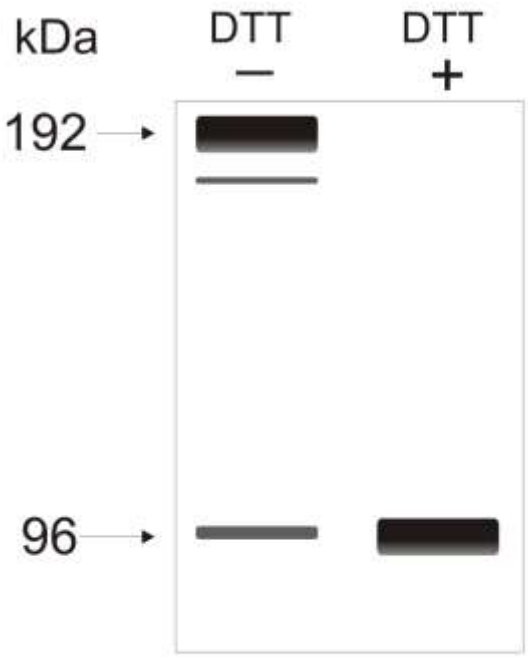


Figure 2: SDS-PAGE analysis of purified glucose oxidase.

圖 2: 利用 SDS-PAGE 分析純化後的葡萄糖氧化酵素。

Taking in account the results obtained from figure 1 and figure 2 the most probable conformation of the glucose oxidase is:

根據圖 1 及圖 2 的實驗結果，下列何者最有可能為葡萄糖氧化酵素的構形？

A) A monomeric enzyme non-glycosylated.

單體, 沒有糖基化的酵素。

B) A monomeric enzyme glycosylated.

單體, 有糖基化的酵素。

C) A homodimer consisting of two monomers both glycosylated.

為同質二元體，兩個單體均有糖基化。

D) A heterodimer consisting of two subunits one of them glycosylated.

為異質二元體，其中一個單體有糖基化。

MARK THE CORRECT ANSWER.

請在正確答案的空格內打乂。

□ A □ B □ C □ D