CHARACTERIZING OF HYPOPHARYNGEAL ENZYMES IN THE DIGESTIC SYSTEM OF HONEY BEES USING GEL ELECTROPHORESIS TECHNIQUES

Julius Pontoh[1] and Nicholas H. Low [2]

[1]*Jurusan Kimia, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Sam Ratulangi, Manado*
[2]*Department of food science University of Saskatchewan, Canada Universitas Sam Ratulangi, Manado*

Diterima 03-09-2009; Diterima setelah direvisi 17-10-2009; Disetujui 24-10-2009

ABSTRACT

Pontoh, J. and N. H. Low, 2009. Karakteristik enzim hipofaring dalam sistem pencernaan lebah madu menggunakan elektroforesis gel.

Keberadaan enzim pencernaan dalam lebah madu memainkan peran yang penting dalam kehidupan lebah. Enzim glukosidase dalam lebah madu diproduksi dalam kelenjar hipofaring dan disekresikan masuk ke dalam system pencernaan. Ketersediaan berbagai teknik elektroporetik membuka ruang lebih besar untuk mengisolasi dan karakterisasi enzim ini sebagai cara baru untuk memahami fungsinya. Tiga sepsis lebah madu yaitu *Apis mellifera*, *A. cerana* dan *A. dorsata*. SDS PAGE digunakan untuk verifikasi efesiensi setiap proses pemurnian protein menjadi protein murni. Kombinasi proses electrophoretic staining and deglycosilation karbohidrat digunakan untuk mengetahui sifat enzim ini. Dua α-glucosidases (1 dan 2) telah dimurnikan menggunakan berbagai tenik kromatografi, tetapi β-glucosidase hanya dapat dimurnikan setelah menggunakan Native PAGE. Massa molekul relatif (M_r's) enzim (α-glucosidase-1, α-glucosidase-2 dan β-glucosidase) dari *A. mellifera* adalah 90, 78 dan 72 kDa. Sedangkan enzim yang berasal dari *A. cerana* adalah 90, 77, dan 82 kDa. Titik isoelektrik (pI) enzim tersebut dari A. mellifera adalah 7.3, 7.5, dan 4.7, sedangkan dalam A. cerana 5.7, 6.2, dan 4.5. Berdasarkan Native IEF-PAGE, α-glucosidase-1 mengandung pita yang lebar, α-glucosidase-2 mengandung hanya pita yang sempit, dan β-glucosidase mengandung beberapa pita. Kombinasi Native PAGE dan IEF PAGE menunjukkan noda glikoprotein yang menunjukkan bahwa α-glucosidase-1 adalah sebuah glikoprotein dengan berbagai heterogenitas mikro.

Keywords : *Apis mellifera*, *A. cerana* dan *A. dorsata*. SDS PAGE, enzim glukosidase

INTRODUCTION

Gel electrophoresis is one of the analytical techniques using in various field of study especially in biochemistry. Characterizing of protein molecules is one of the biochemical technique using intensively up to date. Various electropheretic techniques developed today give numerous combination for characterization any proteins.

Enzymes in digestive systeem have an harsh enveromental circumstances due to the digestive system is the place where all the food consisting of natural substances are being breaking down in to smaller molecules in order to be absorbed into the intestine cells. The breaking agents consist of strong chemicals such as inorganic acid and enzymes.

Proteolitic enzymes are the enzymes breaking up any protein into smaller molecules of amino acids. Due to almost all of the enzymes are proteins they can be subjected to break up by proteolitic enzymes including the proteolitic enzymes themselves can be self destroying. Therefore, many digestive enzymes have various system to protect themselves from activity of digestive proteolitic enzymes.

Honey bees is one of the very economically value insects producing honey. Honey itself is produced from nectar ingested by honey bees, mixed with their saliva and temporary stored in their digestive system. The nectar that turning into honey then secreted into the cells in the hive where the nectar is continue changing into mature honey. During this convertion from nectar to honey, several biochemical changes are processed. Two glucosidases have been proved secreted from hypopharyngel glands (Pontoh and Low, 2002).

The goal of this study is to find out the protecting system of glucosidases in the digestive

Korespondensi dialamatkan kepada yang bersangkutan :
[1]*Jurusan Kimia, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Sam Ratulangi, Manado*
Jl. Kampus Kleak UNSRAT, Manado 95115. Phone : 085256055130, E-mail : -

system in honey bees employing electrophoretic techniques.

METHODOLOGY

Chemicals

All chemicals used in this work were of analytical grade, unless otherwise stated. D-Fructose, D-glucose, sodium dodecyl sulfate (SDS) and sucrose were purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI, USA). Acetic acid, glutaraldehyde, hydrochloric acid, methanol, phosphoric acid, potassium chloride, potassium phosphate, sodium acetate, sodium chloride, sodium hydroxide and sodium phosphate were purchased from BDH (BDH Inc., Toronto, ON). Coomassie brilliant blue (R-250), protein dye solution, standard proteins for isoelectric focussing-gel electrophoresis and standard proteins for sodium dodecyl sulfate-gel electrophoresis were purchased from Bio-Rad Laboratories (Hercules, CA, USA). Sodium carbonate and sodium hydroxide solution (50% w/w) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Ammonium sulfate and bovine serum albumin were purchased from INC Biomedicals Inc. (Aurora, OH, USA). Bromphenol blue (3,3′,5,5′-tetrabromophenolsulfonphthalein) was purchased from J.T.Baker Chemical CO. (Phillipsburg, NJ, USA). Glycoprotein staining kit was purchased from Pierce Chemical Company (Rockford, IL, USA). The kit contained GelCode® glycoprotein stain, oxidation reagent (periodic acid), reduction reagent (sodium metabisulfite), positive control protein (horseradish peroxidase) and negative control protein (soybean trypsin inhibitor). Modified trypsin from porcine pancreas was purchased from Promega (Madison, WI, USA). Aprotinin, ethylenediaminetetraacetic acid disodium salt (EDTA), leupeptin, N-glycosidase F deglycosylation kit, neuraminidase, O-glycosidase, Pefabloc®SC and pepstatin were purchased from Roche Diagnostics GmbH (Mannheim, Germany; formerly: Boehringer Mannheim GmbH). The N-glycosidase F deglycosylation kit contained peptide-N-glycosidase F (PNGase F), denaturation buffer, control glycoproteins (human transferin, ribonuclease B and human transferin α_1-acid glycoprotein), reaction buffer (pH 7.2) and standard relative molecular mass (M_r) protein markers. Acetonitrile, acrylamide/bis-acrylamide (30% solution), 6-aminohexanoic acid - Sepharose 2B, *p*-aminophenyl α-D-glucopyranoside, *p*-aminophenyl β-D-glucopyranoside, ammonium bicarbonate, ammonium persulfate, andrenocorticotropic hormone (ACTH), des-Arg Bradykinin, carbodiimide, cellobiose, α-cyano-4-hydroxycinnamic acid, formaldehyde, glycerol, maltose, 2-mercaptoethanol, *p*-nitrophenyl-α-D-glucopyranoside (α-PNPG), *p*-nitrophenyl-β-D-glucopyranoside (β-PNPG), phenylmethanesulfonyl fluoride (PMSF), potassium ferricyanide, protease (from *Streptomyces griseus*), silver nitrate, sodium thiosulfate, N,N,N′,N′-tetramethylethylenediamine (TEMED), trichloroacetic acid, trifluoroacetic acid and tris(hydroxymethyl)aminoethane were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Water obtained from a Milli-Q water purification system was used to prepare all solutions and to rinse gels and is designated as ultrapure water in this thesis (Millipore Corporation, Bedford, MA, USA).

Materials

Three honey bee species, *Apis mellifera*, *A. cerana* and *A. dorsata*, were used in this study. These honey bees were killed on site by treatment with dry ice. All collected honey bees were stored at -30 °C until used for enzyme extraction. In general, the collected honey bees used for enzyme extraction were adult workers based on fore wing size (*A. mellifera* ~9 mm; *A. cerana* ~8 mm; *A. dorsata* ~12 mm).

Each frozen (-30 °C) honey bee was cut to remove the head and abdomen from the discarded thorax. After thawing (5 °C), the abdomen was dissected by peeling off both dorsal and ventral plates with two pairs of # 5 Dumont forceps (A. Dumont & Fils; Autils, Switzerland) to expose the honey sac and ventriculus. The honey sac was then removed by serving the proventriculus behind it and placed in a glass test tube (13 x 100 mm). The exposed ventriculus was then disconnected from the hindgut (Figure 2) and placed in a separate test tube. The hypopharyngeal glands were isolated by first breaking the occiput at the back of the head, and then the glands were removed by forceps. All dissections were performed using a stereomicroscope (WILD™, Heerbrugg, Switzerland).

Approximately 250 honey bees were required for glucosidase isolation from the ventriculus, and approximately 1,000 honey bees

were required for glucosidase isolation from the honey sac and hypopharyngeal glands.

Native PAGE (Polyacrylamide Gel Electrophoresis)

Native PAGE was performed at high pH (9.5) with a discontinuous gel system consisting of a 4% polyacrylamide stacking gel and a 7.5% polyacrylamide separating gel (Bollag and Edelstein, 1991).

Fractions (~2.0 mL) containing α- and β-glucosidase activities from Superose® 12 chromatography for the purification of α- and β-glucosidases from the ventriculus of *A. cerana* were placed in 10 microcentrifuge tubes (500 μL) and freeze-dried by Hetovac freeze dryer. Ten microliters of 310 mM Tris-HCl buffer pH 6.8 containing glycerol (50% v/v) and bromphenol blue (0.05% v/v) were added to each tube. Samples were then individually loaded onto a native gel. The gel was run in a 150 mV electric field for 90 minutes.

The low affinity of glucosidase toward coomassie brilliant blue stain after native PAGE was overcome by the use of modified silver staining for glycoproteins (Dubray and Bezard, 1982). Modification of the method was required and involved treatment of the gel with a solution containing 25% ethanol and 10% acetic acid for 10 – 12 hours followed by 7.5% acetic acid for 30 minutes at room temperature. The gel was then transferred to a 0.2% aqueous periodic acid solution for 1 hour at 4 ^{o}C. After intensive washing with ultrapure water, the gel was transferred into a 0.25% silver nitrate solution for 30 minutes at room temperature. The gel was immersed in ultrapure water for 3 minutes and was then transferred to a developing solution containing 2.5% sodium carbonate and 0.02% formaldehyde until the glycoproteins developed a gray/black colour at room temperature. Colour development was terminated by transferring the gel to a 5% acetic acid solution at room temperature.

Glucosidases were localized on the unstained gel by excising into 2 mm bands which were then individually assayed for α- and/or β-glucosidase activities following the standard method (Pontoh and Low, 2002). Fractions containing α- or β-glucosidase activities (Pontoh and Low, 2002) were separately collected for further analysis. These collected samples were assigned as purified α-glucosidase-2 and β-glucosidase from the ventriculus of *A. cerana*.

Sodium Dodecyl Sulfate (SDS)-PAGE

Samples containing protein ranging from 0.1 to 15 μg were transferred into a microcentrifuge tubes (500 μL) and freeze-dried. Sample buffer (10 μL) of 125 mM Tris-HCl buffer pH 6.8 containing glycerol (10% v/v), SDS (2% w/v), mercaptoethanol (ME; 5% v/v) and bromphenol blue (0.01% w/v) was added to each microcentrifuge tube (500 μL) and the resulting protein solution was denatured by heating in boiling water for 4 minutes. After centrifugation (Sorvall SS-1 Superspeed Angle Centrifuge; Ivan Sorvall Inc., Norwalk, CT, USA) for 10 minutes at 3500 x g, samples were placed in comb wells.

For discontinuous SDS-PAGE, two different separation gels were used: (a) a 4% polyacrylamide stacking gel (pH 6.8) was overlaid on top of a 7.5% polyacrylamide separating gel (pH 8.3). The gel was placed in a 200 mV electric field and run for 35 minutes; (b) a gradient (4-20%) polyacrylamide gel (Ready Gels, Bio-Rad Laboratories, Hercules, CA, USA; pH 8.3) run in a 200 mV electric field for 50 minutes.

Proteins were stained with coomassie brilliant blue or silver stain. The coomassie brilliant blue staining was performed following the method described by Bollag and Edelstein (1991). Silver staining was accomplished by fixing the gel in 45% methanol containing 10% acetic acid, or after coomassie brilliant blue staining. The gel was then transferred to a solution containing 50% ethanol and 10% acetic acid for approximately 2 hours, and finally to a solution containing 30% ethanol, 4% sodium acetate, 0.5% glutaraldehyde and 0.2% sodium thiosulfate for 1 hour. After washing with ultrapure water, the gel was immersed for 30 minutes in a solution containing 0.25% silver nitrate containing 0.02% formaldehyde. After washing with ultrapure water, silver stained proteins in the gel were developed by immersion in a solution of 2.5% sodium carbonate and 0.02% formaldehyde until the proteins became gray/black. Colour development was terminated by transferring the gel to a 5% acetic acid solution.

Deglycosylation and Glycoprotein Staining

Deglycosylation of isolated honey sac glucosidases was performed using a N-glycosidase F Deglycosylation Kit alone or in combination with neuraminidase and O-glycosidase following the manufacturer's

recommended protocol (Roche Diagnostics GmbH). Purified glucosidase samples containing 1 to 10 μg protein were transferred into a microcentrifuge tube (500 μL; Eppendorf, Hamburg, Germany) and freeze dried. Denaturation buffer (5 μL; 125 mM Tris-HCl buffer pH 6.8) containing glycerol (10% v/v), SDS (2% w/v), mercaptoethanol (ME; 5% v/v) and bromphenol blue (0.01% w/v) was added into the tube and heated in boiling water for 3 minutes. After centrifugation (Sorvall SS-1 Superspeed Angle Centrifuge; Ivan Sorvall Inc., Norwalk, CT, USA) for 10 minutes at 3500 x g, the mixture was transferred into a new microcentrifuge tube containing 10 μL of reaction buffer. A 10 μL aliquot of N-glycosidase F (2 units) or a mixture of N-glycosidase F, neuraminidase (2 milli-units) and O-glycosidase (2.5 milli-units) was then added, and the mixture was heated for 6 hours at 37 ^{o}C in a water bath (Haake, Mess-Technik GmbH, Karlsruhe, Germany). A control glycoprotein mixture containing horseradish peroxidase and soybean trypsin inhibitor was run in conjunction with the samples. A second sample and control glycoprotein mixture were also prepared without deglycosylation. The effectiveness of protein deglycosylation was evaluated by analyzing the reaction mixture by SDS-PAGE in conjunction with glycoprotein staining followed by coomassie brilliant blue or silver staining.

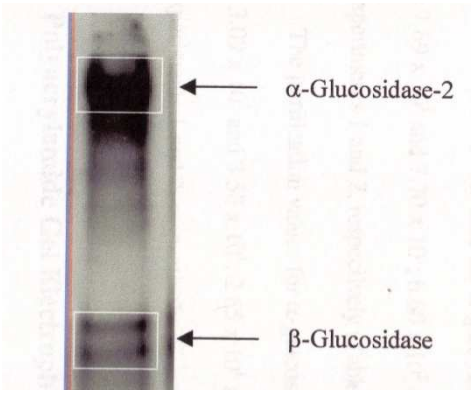
Glycoproteins were stained using a GelCode® Glycoprotein Staining Kit following the suggested protocol of the manufacturer (Pierce Chemical Company, Rockford, IL, USA). The protocol: following protein separation by SDS-PAGE, the gel was fixed by complete immersion in 100 mL of 50% methanol for 30 minutes, followed by gentle agitation with 100 mL of 3% acetic acid for 10 minutes. The gel was then washed twice with 3% acetic acid for 5 minutes, and was transferred into 25 mL of oxidation solution containing 1% periodic acid in 3% acetic acid and gently agitated for 15 minutes. The gel was then washed three times with 100 mL of 3% acetic acid for 5 minutes, and was transferred into 25 mL of GelCode® glycoprotein staining reagent and gently agitated for 15 minutes. The gel then was transferred into 25 mL of reduction reagent containing sodium metabisulfite and gently agitated for a total of 5 minutes each. The gel was washed with 3% acetic acid for 10 minutes and then with ultrapure water. Glycoproteins were observed as magenta bands.

RESULTS AND DISCUSSION

Native-PAGE (Polyacrylamide Gel Electrophoresis)

Initial experiments using native electrophoresis for the separation of α-glucosidase from β-glucosidase were limited because these enzymes were not effectively stained by coomassie brilliant blue. In order to overcome this difficulty, a modified silver-staining technique specific for glycoprotein visualization (Dubray and Bezard, 1982) was used for all subsequent native gel electrophoresis experiments.

Fractions with β-glucosidase activity were reduced in volume and subjected to native gel electrophoresis. Native gel electrophoresis afforded the separation of α-glucosidase-2 from β-glucosidase (Figure 1).

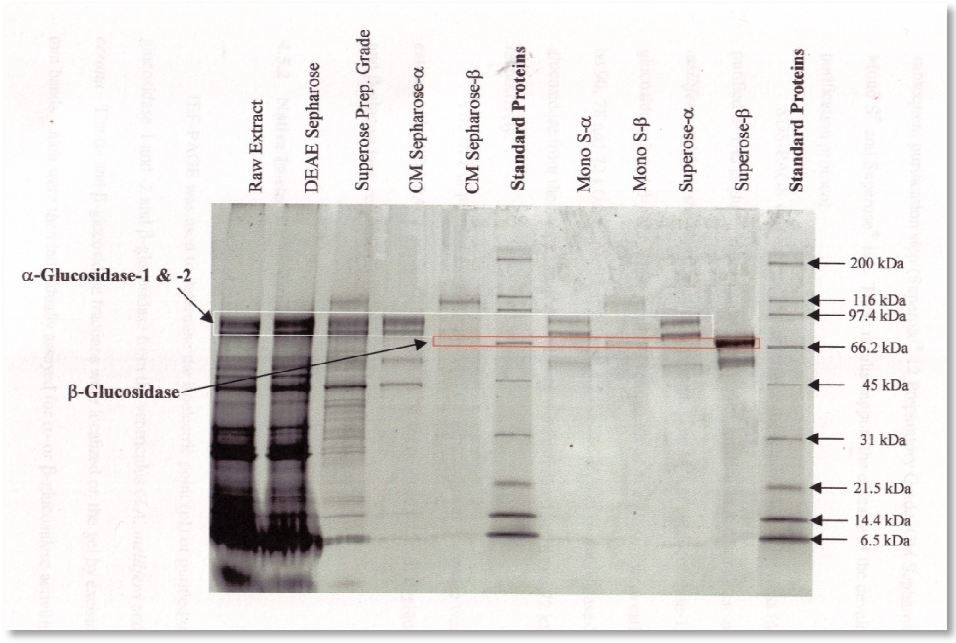


Gambar 1. Native PAGE for of the Superose-12 gel filtrations containing α-glucosidase-2 and β-glucosidase from the ventriculus of A. cerana (modified silver stained)

Sodium Dodecyl Sulfate (SDS)-PAGE

SDS-PAGE was used to monitor α- and β-glucosidase purification from the ventriculus of *A. mellifera* during the course of protocol development with the exception of the hydroxyapatite purification step (Figure 2). Although the total protein content between the raw and DEAE chromatographic stage dropped significantly (by approximately 42%), their profiles were similar (Figure 2, lanes 1 and 2). However, dramatic changes in protein patterns coupled with an increase in specific activity for both α- and β-glucosidases were observed in each subsequent purification step (Pontoh and Low, 2002). These results support the efficacy of the developed purification protocol.

SDS-PAGE was also used to determine the relative molecular mass (M_r) of purified α- and β-glucosidases extracted from the ventriculus and honey sac of *A. mellifera* and from the ventriculus of *A. cerana*. The M_r's of α-glucosidase-1, α-glucosidase-2 and β-glucosidase from the ventriculus of *A. mellifera* were estimated to be 90, 78 and 72 kDa, respectively (Figure 3). The M_r's of α-glucosidase-2 and β-glucosidase from the honey sac of *A. mellifera* were estimated as 78 and 72 kDa, respectively.



Gambar 2. SDS PAGE of α- and β-glucosidases from the ventriculus of A. mellifera before and after each identified purification step.

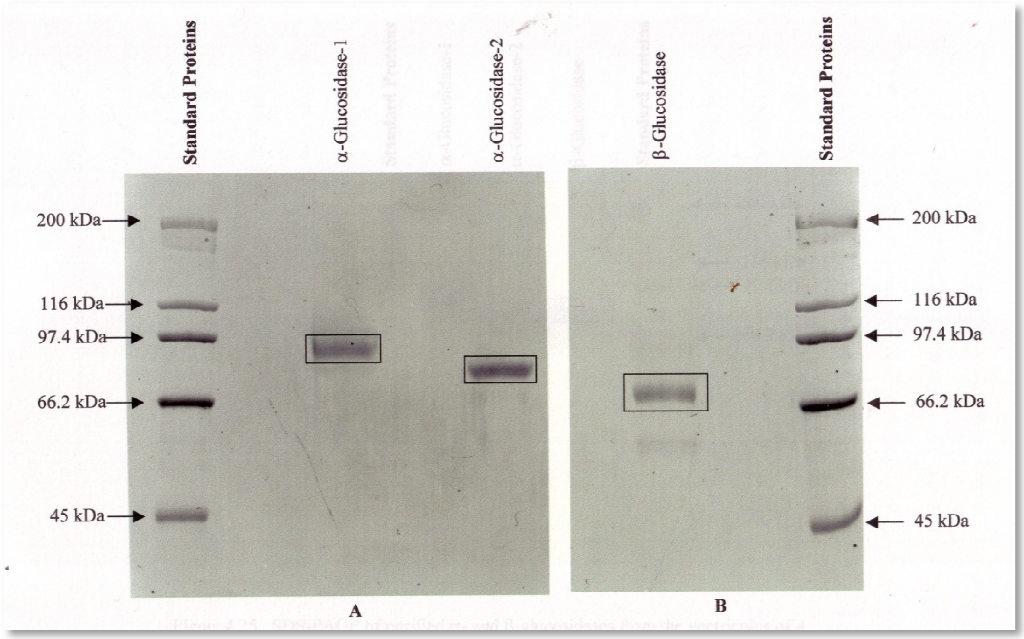


Figure 3. SDS PAGE of purified α-glucosidases (A) and purified –glucosidase from the ventriculus of *A. mellifera* stained with commassie blue technique. The Mr's of α-glucosidase-1, α-glucosidase-2 and β-glucosidase were estimatede as 90, 78 and 72 kDa.

The M_r's of purified glucosidases from the ventriculus of *A. cerana* were estimated by SDS-PAGE to be 90, 77 and 82 kDa for α-glucosidase-

1, α-glucosidase-2 and β-glucosidase, respectively (Figure 4). The different between β-glucosidases from *A. melifera* and *A. cerana* (72 and 82 kDa, respectively) was significant. The different could be due to the present of oligosaccharides in both those disgestive enzymes.

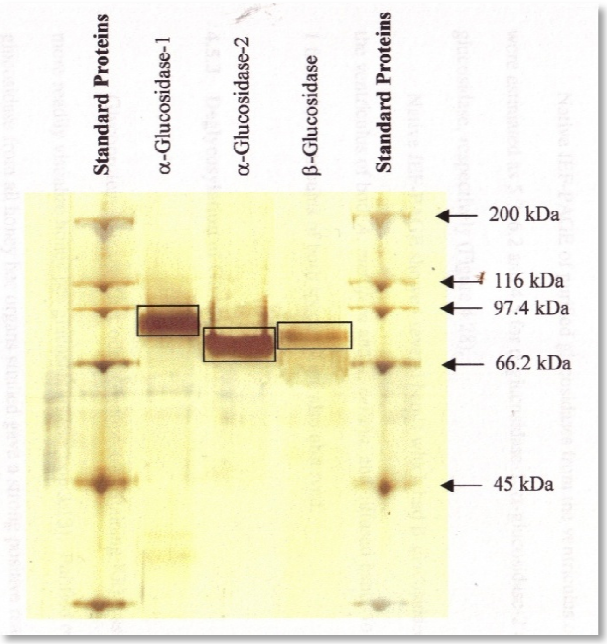


Figure 4. SDS PAGE of purified α and β-glucosidases from the ventriculus of *A. cerana* (silver stained). The Mr's of α-glucosidase-1, α-glucosidase-2 and β-glucosidase were estimatede as 90, 77 and 82 kDa.

Native Isoelectric Focussing (IEF) PAGE

IEF-PAGE was used to determine the isoelectric point (pI) of purified α-glucosidase-1 and -2 and β-glucosidase from the ventriculus of *A. mellifera* and *A. cerana*. The α- and β-glucosidase fractions were localized on the gel by excising into 2 mm bands which were then individually assayed for α- or β-glucosidase activities.

Results showed that α- and β-glucosidase activities corresponded to protein bands (Figure 5). Based on electrophoretic mobility comparison to standards, the pI's of α-glucosidase-1 and -2 from the ventriculus of *A. mellifera* were estimated to be 7.3 and 7.5, respectively (Figure 5.A). Employing the same methodology, the pI of β-glucosidase purified from the ventriculus of *A. mellifera* was estimated to be 4.7 (Figure 5.B).

Native IEF-PAGE of purified glucosidases from the ventriculus of *A. cerana* were estimated as 5.7, 6.2 and 4.5 for α-glucosidase-1, α-glucosidase-2 and β-glucosidase, respectively (Figure 6).

Native IEF-PAGE showed several bands which had β-glucosidase activity from the ventriculus of both *A. mellifera* and *A. cerana*, and diffused bands for α-glucosidase-1 from the ventriculus of both species were also observed.

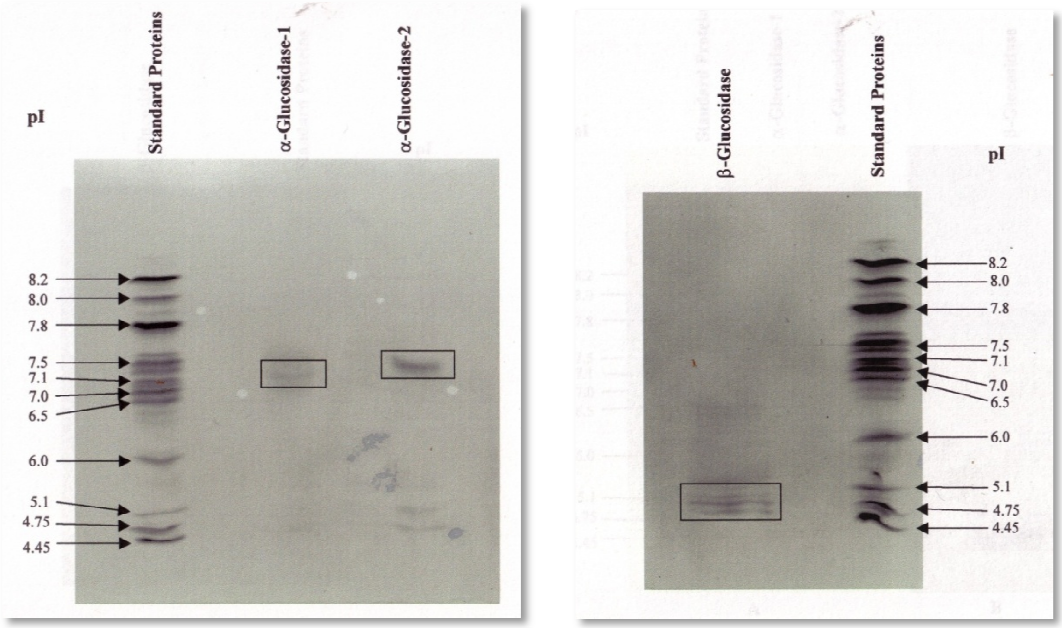


Figure 5. Native IEF-PAGE of purified α-glucosidases (A) and β-glucosidase (B) from the ventriculus of *A. melifera* (silver stained). The *p*I of α-glucosidase-1 and α-glucosidase-2 were 7.3 and 7.5, respectively and the of β-glucosidase was 4.5.

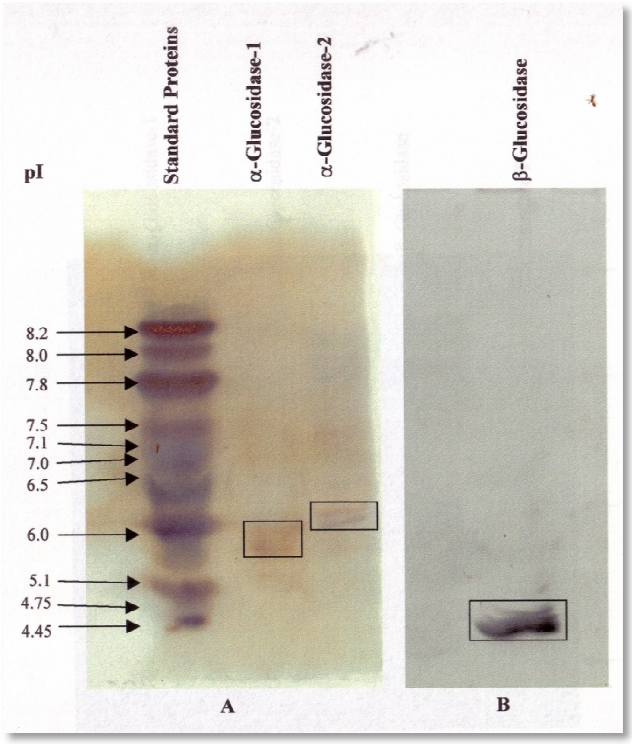


Figure 6. Native IEF-PAGE of purified α-glucosidases (A) and β-glucosidase (B) from the ventriculus of *A. melifera* (silver stained). The *p*I of α-glucosidase-1 and α-glucosidase-2 were 7.3 and 7.5, respectively and the of β-glucosidase was 4.5.

Deglycosylation of Glucosidase

Glycoprotein staining (GelCode® Glycoprotein Staining Kit) was introduced to more readily visualize honey bee glucosidases. Purified α- and β-glucosidase from all honey bee organs studied gave a strong positive reaction to glycoprotein staining after SDS-PAGE (Figure 7).

In order to obtain a more accurate representation of these enzymes for peptide mass mapping by MALDI-TOF-MS, deglycosylation with N-glycosidase F was investigated. Treatment of α-glucosidase-1 from the ventriculus of *A. mellifera* with N-glycosidase F reduced the M_r of this enzyme (Figure 7.A, Lane 2 and 3).

The results showed that after deglycosylation the glucosidase still had a positive reaction to glycoprotein staining indicating that some of the glycan was not hydrolyzed (Figure 7.B, Lane 2 and 3). Further treatment of α-glucosidase-1 with O-glycosidase and neuraminidase did not appear to change its M_r (Figure 7.A, Lane 4 and 5) and glycoprotein staining still gave a positive reaction (Figure 7.B, Lane 4 and 5). Treatment of α-glucosidase-1 with a combination of N-glycosidase F, O-glycosidase and neuraminidase did not result in an M_r which could be distinguished from N-glycosidase-F treated α-glucosidase-1 (Figure 7.A, Lane 7 and 8). Glycoprotein staining gave a positive reaction for both enzymatic treatments (Figure 7, Lane 7 and 8). The same results were observed for α-glucosidase-2 from the ventriculus of *A. mellifera* treated with these enzymes.

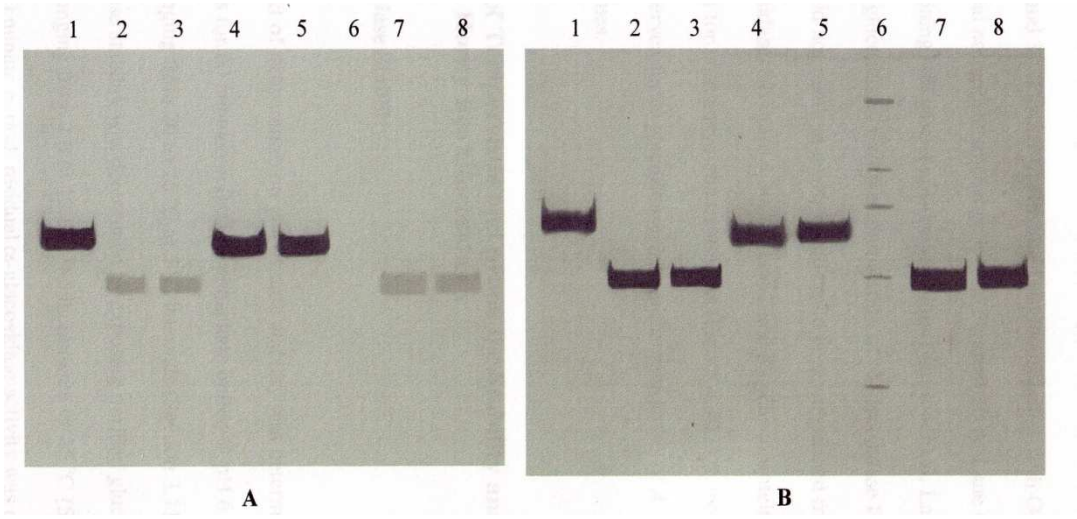


Figure 7. SDS-PAGE of purified α-glucosidase-1 from the ventriculus of *A. mellifera*. A, glycoprotein stained and B, coomassie brilliant blue stained. Lane 1 undeglycosylated, 2 & 3 deglycosylated with N-glycosidase F, 4 & 5 deglycosylated with O-glycosidase and neuraminidase, 6 standard proteins, 7 & 8 deglycosylated with N-glycosidase F, O-glycosidase and neuraminidase.

Discussion

The relative molecular masses of α-glucosidase-1 from the ventriculus of *A. mellifera* and *A. cerana* were both found to be 90 kDa by SDS-PAGE. These results agree with those reported by Huber (1975) and Takewaki, et al. (1980) for α-glucosidase-1 isolated from whole honey bees of *A. mellifera*.

The M_r's of α-glucosidase-2 from the ventriculus and honey sac of *A. mellifera* and from the ventriculus of *A. cerana* were found to be 78, 78 and 77 kDa, respectively by SDS-PAGE. These results agreed with those reported by Huber and Mathison (1976) and Takewaki, et al. (1980) on α-glucosidase-2 isolated from whole honey bees (*A. mellifera*) which were 78 and 76 kDa, respectively. Takenaka and Echigo (1976; 1978) reported an M_r of 73 kDa for this same enzyme from honey. Kubo, et al. (1996) also reported an M_r of 70 kDa for α-glucosidase from the hypopharyngeal glands of *A. mellifera*. The range (70-78 kDa) observed for this enzyme from these sources may be due to slight band migration/calculation of and/or the microheterogeneity of α-glucosidase-2. Microheterogeneity of glycoproteins is defined as the diversity of carbohydrate composition, arrangement and attachment site on the glycoprotein backbone (Vliegenthart and Montreuil, 1995). Mertz, et al. (1996) found that glycosylation effects protein migration during SDS-PAGE analysis.

The M_r of β-glucosidase from both the ventriculus and honey sac of *A. mellifera* was found to be 72 kDa, however, β-glucosidase from the ventriculus of *A. cerana* had a M_r of 82 kDa. These results indicate that the β-glucosidase present in the ventriculus of these two species is different.

Comparison of the M_r's of α- and β glucosidases isolated from selected organs of *A. mellifera* and *A. cerana* to their gel filtration chromatographic elution profiles indicates that each of these enzymes consists of a single sub-unit (monomer).

The pI for α-glucosidase-1 from the ventriculus of *A. mellifera* was found to be 7.3 which was identical to that reported by Huber (1975). The pI of α-glucosidase-1 from the ventriculus of *A. cerana* was found to be 5.7. Possible reasons for these differences could be the composition, arrangement and glycosylation sites of the specific carbohydrate moieties on the protein (microheterogeneity).

The pI of α-glucosidase-2 from the ventriculus of *A. mellifera* was found to be 7.5 and that from the ventriculus of *A. cerana* was found to be 6.0. Huber and Mathison (1976) reported that the pI of this enzyme from whole honey bees was 6.5. Possible reasons for these differences are the same as those presented for α-glucosidase-1.

The pI of β-glucosidase from the ventriculus of *A. mellifera* and *A. cerana* was

found to be 4.7 and 4.5, respectively. This is the first reported pI of β-glucosidase from any honey bee organ or species.

During IEF-PAGE analysis of purified α-glucosidase-1 from the ventriculus of *A. mellifera* and *A. cerana*, it was noted that the bands were diffuse (~2.5 mm in width). The IEF-PAGE of α-glucosidase-2 from the ventriculus of *A. cerana* and β-glucosidase from the ventriculus of *A. mellifera* and *A. cerana* all showed the presence of several bands. These diffused and multiple bands could result from the microheterogeneity of these glucosidases. Packer, et al. (1999) reported that 2-D PAGE analysis of glycoproteins often result in "trains" of spots that have different pI's and/or M_r's. The analysis of porcine pancreatic ribonuclease by IEF-PAGE shows that the different pI's observed were due to the carbohydrate composition, arrangement and attachment site on the protein backbone (Scheele, 1975; Rudd, et al., 1994).

CONCLUSION

Gel electrophoretic techniques are still the very powerfull techniques to reveal the properties of proteins and enzymes. The developments of advance techniques in stainning and nature enzyme isolation were able to reval the presence and properties of pure glucosidases from various parts of honey bees.

The combination of gel electrophoretic and deglycosilatic enzymes was able to reveals the more detailed properties of glycoprotein such as α-glucosidase-1 from digestive system in honey bees.

The purified enzymes from gel electrophoresis can be used also for proteomic analysis with mass spectrometic techniques (Pontoh, 2009).

REFERENCES

Bollag, D.M. and S.J. Edelstein. 1991. "Protein Methods". Wiley-Liss, New York, NY.

Dubray G. and G. Bezard. 1982. A highly sensitive periodic acid-silver stain for 1,2-diol groups of glycoproteins and polysaccharides in polyacrylamide gels. Anal. Biochem. 119:325-329.

Huber, R.E. 1975. The purification and study of a honey bee abdominal sucrase exhibiting unusual solubility and kinetic properties. Arch. Biochem. Biophys. 168:198-209.

Huber, R.E. and R.D. Mathison. 1976. Physical, chemical and enzymatic studies on the major sucrase of honey bees (*Apis mellifera*). Can. J. Biochem. 54:153-164.

Kubo, T., M. Sasaki, J. Nakamura, H. Sasagawa, K. Ohashi, H. Takeuchi and S. Natori. 1996. Change in the expression of hypopharyngeal-gland proteins of the worker honeybees (*Apis mellifera* L.) with age and/or role. J. Biochem. 119:291-295.

Mertz, E., T. Sareneva, S. Haebel, I. Julkumen and P. Roepstorff. 1996. Mass spectrometric characterization of glycosylated interferon-d variants seperated by gel electrophoresis. Electrophoresis. 17:925-931.

Packer, N.H., M.S. Ball and P.L. Devine. 1999. Glycoprotein detection of 2-D separated proteins. In: "Methods in Molecular Biology, Volume 112: Proteome Analysis Protocols", A.J. Link (ed). Humana Press Inc., Totowa, NJ. pp 112:341-352.

Pontoh, J. and N.H. Low. 2002. Purification and characterization of b-Glucosidase from honey bees (Apis mellifera). Insect Biochemistry and Molecular Biology 32:679-690.

Simpson, J., I.B.M. Riedel and N. Wilding. 1968. Invertase in the hypopharyngeal glands of the honeybee. J. Apic. Res. 7:29-36.

Takenaka, T. and T. Echigo. 1976. The properties and transglucosidation action of α-glucosidase of honey. Bull. Fac. Agr. Tamagawa Univ. 16:1-9.

Takenaka, T. and T. Echigo. 1978. Properties and transglucosidation action of α-glucosidase of honey (Part 2). Bull. Fac. Agric. Tamagawa Univ. 18:22-31.

Takewaki, S., S. Chiba, A. Kimura, H. Matsui and Y. Koike. 1980. Purification and properties of α-glucosidases of the honey bee *Apis mellifera* L. Agric. Biol. Chem. 44:731-740.

Vliegenthart, J.F.G. and J. Montreuil. 1995. Primary structure of glycoprotein glycans. In: "Glycoproteins", J. Montreuil and J.F.G. Vliegenthart (eds). Elsevier, New York, NY. pp 13-28.