Characterization of Invertase from *Saccharomyces cerevisiae*

Experiment 6: Characterization of Invertase Using SDS-PAGE

Experiment 6 Introduction

The method Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE technique) used on experiment 3 works well on detecting any different sizes of protein. This is expressed in kiloDaltons (kD) ranging from 200kD with the largest molecular weight down to 2.5 kD with the smallest molecular weight. SDS-PAGE would not only help us to separate sample based on molecular weight and polypeptide length but it also helps us to visualize how many subunits the invertase has.

SDS-PAGE reliability to work with enzymes is due to its composition of acrylamide which is a neurotoxin and polymerizes in the absence of oxygen. We initiate polymerization by adding freshly prepared 10% ammonium persulfate (AP) followed by N,N'-tetramethylenediamine (TEMED).

Sodium dodecyl sulfate (SDS) is compound with detergent properties is capable to associate with the protein in this case an enzyme and denature it and it gives an overall negative charge.

The negative charge will destroy most the tertiary and secondarystructure, which are strongly attracted to toward an anode (positively-charged electrode) in an electric field. Sample buffer contains beta–mercaptoethanol which breaks disulfide bonds so more bands on the gel can be seen.

0.5 % Coomassie blue dye penetrates the entire gel and sticks permanently to the proteins.

Experiment 6 Procedure

Insert the comb between glass plates. The running or separation gel should be one centimeter below the end the comb. Remove the comb and pour the solution below with the help of p-1000. Wear glove during the preparation of running and stacking gel because 30 % acrylamide/ 0.8 % bis is neurotoxin.

Prepare separation gel solution:	(Amount for 2 gels)
1. Distill water	3.125 ml
2. 1.5 M Tris-HCl/0.4 % SDS	1.875 ml
3. 30 % acrylamide/ 0.8% Bis	2.5 ml
4. 10 % APS	2.5 µl
5. TEMED	5.0 µl

Mix all solutions gently and fill it up to the line marked previously. Cover the solution the with water-saturated butanol to even out the edges. Wait about 10-20

minutes, the butanol should be removed by tilting and rinsing with distilled water about 2 to 3 times. Towel paper should be use to dry the glass. Stacking gel should be added on top of the running gel by mixing the following solution.

Prepare stacking solution:

1. Distill water	1.525 ml
2. 0.5 M Tris-HCl / 0.4% SDS	0.625 ml
3. 30 % Acrylamide/ 0.8 % Bis	0.325 ml
4. 10% APS	12.5 µl
5. TEMED	2.5 µl

Place comb at a slight angle. Allow gel to polymerize for about 5 to 10 minutes. After gel has polymerized, place the glass with the running and stacking gel inside a mini PAGE chamber filled with tank buffer until the well are completely filled with the buffer.

Prepare tank buffer:

5 X SDS

Tris	15.0 grams
SDS	5.0 grams
Glycine	72.0 grams

Dilute 100 ml of 5x SDS-PAGE into a Liter to make 1X SDS tank buffer.

Invertase, sample buffer, marker should be kept on ice.

Prepare samples:

- High concentration of sample invertase (21 µl) and low concentration of sample buffer (7 µl). Well #3.
- Low concentration of sample invertase (10µl) and high concentration of sample buffer (7 µl) and autoclaved distilled water (10µl). Well # 4
- Control Protein (21 µl) and sample buffer (7 µl). Well #5.
- Concentrated Invertase (28 µl)
- Marker (7 µl)

Sample buffer contains distilled water, 0.5 M Tris-HCl (1.0 ml), Glycerol, 10% SDS, beta-mercaptoethanol, bromophenol blue.

Before placing sample mixtures of invertase in each well, it should be boiled at 95 C for 4 minutes in order to denature it.

Place the lid and electrophorese at 150 volts for around two hours.

Turn off the power, remove lid and pour off the tank buffer.

Remove gel and place it into container with enough 0.5 % Coomassie blue dye for almost 30 minutes.

Remove Coomassie blue dye (staining solution) and add enough destaining solution.

Aid removal of the dye with Kimwipes or sponge.

Destaining complete, place gel on a piece of clear plastic overhead film.

Reference: Ryoko Yanamoto, M.S.