Characterization of Invertase from *Saccharomyces cerevisiae*

Experiment 6: Characterization of Invertase Using Agarose Gel Electrophoresis

Experiment 7 Introduction

Agarose gel electrphoresis worked well in experiment 6. Agarose is more porous than acrylamide. The gel is placed in a tank with an ionic buffer. An agarose gel has larger pores than a polycrylamide gel, enabling it to analyze proteins of much larger mass than a polycrylamide gel.

Molecules can be separated by size and charge using a method known as agarose gel electrophoresis. It is one of the most common methods used by researchers to isolate and identify fragments. Agarose forms a matrix that acts as a sieve through which smaller molecules can migrate faster than larger ones.

Experiment 7: Procedure

Pour 60 ml of 2XTAE into 100 ml beaker. Add 0.36 grams of agarose. Beaker, buffer solution and agarose should be weighted before it is heated in the microwave for over a minute. The solution is ready when it is completely clear. The beaker should be handled carefully. Reweigh and add water to the original mass. Pour the solution into casting tray and insert comb, which will leave wells. Wait until the mixture has solidified and remove comb carefully.

Prepare Agarose buffer:

50 X TAE Tris 96.8 grams
Acetic acid 22.84 ml
0.5M EDTA 40 ml (pH 8.0)
Dilute 50X TAE to 2 X TAE
Add up to 400 ml and autoclave.

Prepare 2XTAE
(1/25) ~ 240 ml H2O and 10 ml 50x TAE

Invertase, sample buffer, marker should be kept on ice.
Prepare samples:

- Marker (7μl). Well # 2.
- Invertase (20 μl). Well # 4.
- High concentration of sample invertase in distilled water (15μl invertase and 5μl distilled water). Well #5.
- Low concentration of invertase # 1(5μl of invertase, 8 μl distilled water and 7 μl of sample buffer). Well # 7.

- Low concentration of invertase #2 (5μl of invertase, 7 μl distilled water and 8 μl of sample buffer). Well #9.

Place the casting tray inside the electrophoresis cell. Fill the electrophoresis cell with 2XTAE until the casting tray has been completely submerged. Insert the samples above in each well.
Connect the power cables to the cell. Turn it on and set voltage to 150 volts
Turn off the power when the standard marker has moved within 1 cm of the end.
Slide gel into staining solution (Coomasse blue dye).
Leave it for a period of about 5-10 minutes. Destain gel by adding 100 ml of destaining solution .
Aid removal with of the dye with Kimwipes or sponge.
Destaining complete, place gel on a pice of clear plastic overhead film.

Conclusion

SDS-PAGE helps to visualize the size of the invertase. On the other hand, agarose is going to separate invertase by charge and size. Addition of Beta-Mercapentanol in the sample buffer should help to break the disulfide bonds. After running the SDS-PAGE, we were able to notice that the bands in each lane are identical. Concentrated Invertase and different amount of sample buffer added to the invertase showed us that addition of beta-mercapentanol do not affect invertase because it does not have disulfide bonds. Bands were seen at 50, 37, 25 kD in all four samples.

On agararose gel electrophoresis the pI was different for all 3 samples. Low concentration of invertase and beta-mercapentanol increases the pI. We can conlude that diluted invertase travel further and beta-mercapentanol may not have influence the distance traveled because the SDS-PAGE which is acrylamide