Daniel Auerbach

Abstract

Introduction:

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Intertidal organisms are often exposed to drastic temperatures changes multiple times a day. As tides flow in and out of coastal areas, these animals are exposed to direct heat, or rapid warming of water because of the smaller volume around them. To cope with these changes several species have adapted different strategies in order to avoid desiccation and ultimately death. Shelled species use suction in order to hold water so that they do not dry out. Anemones can curl into their body cavity to reduce surface area, and more of their body being directly in the heat. For more mobile animals such as sea stars and sea urchins, they use their tube feet in order to avoid desiccation all together. As the tides begin to flow out, they are able to slowly remove themselves from areas that would be directly in exposed to the heat of the sun. Usually this means they move into cracks or into the water of the tide pools.

Although these intertidal species have mechanisms in order to prevent injuries from heat, over exposure can damage proteins. Because of this heat shock proteins are available in every cell in order to carry the damaged proteins to break them down. They also release the chaperone to the heat shock factor in order to produce more heat shock protein. The heat shock protein is also considered a “stress protein” because of the up regulation when species are in unfavorable conditions. Desiccation as well as overexposure are two stressors that will create the mass production of these heat shock proteins.

Because the intertidal species are so susceptible to conditions not ideal for living, they would be more likely to create heat shock proteins and faster than other species. Although they have been able to adapt to these extreme condition and have fair warning with changing tides, still they may be stuck in conditions that they should not be in. With our experiment we will be looking to see how heat shock production changes when put directly into heat and unfavorable conditions. With little water and higher temperatures than normal, we would expect to see much faster and greater amounts of heat shock proteins, in order to cope with the conditions they are in.

Materials and Methods:

Our experiment took place at South Alki beach outside of Seattle, WA. We collected approximately 15 urchins but only 4 were used in our actual experiment. We selected two of the biggest ones for control, and two for the experiment. The treatment urchins were exposed to two hours of heat (approximately 23 degrees C) in minimal water (around 2 cm) for seven consecutive days. Heat was issued to the urchins by pulling them out of the water and under heat lamps, and then when treatment was finished they were put back directly into their tank. Tissue samples were collected at the beginning of experiment and at the end by pulling tube feet from the four urchins used.

Protein extraction was next in the lab. 500 ul of CellLytic MT solution was added to the mL snap cap tube with our tissue. We then homogenized and inverted the tubes before centrifuging (find name of centrifuge) them from 10 minutes at maximum speed. The final step in the extraction protocol was to transfer the supernatant (all clear liquid except for the pellet at the bottom of tube) into a new snap cap tube. These tubes were labeled and put back onto ice for protein quantification.

In 2 mL screw cap tubes, a 1:2 dilution was made with the protein sample and 0.1% ETOH water. The final tubes had 15 mL of total dilution. ATTATCH TABLE FOR DILUTIONS. A final screw cap tube was filled with 30 mL of water in order to serve as a blank. 1.5 mL of Bradford agent (get exact name of Bradford agent) was added to all of the test tubes, and then the tubes were inverted and incubated at room temperature for ten minutes. These tubes were then transferred into clean cuvettes and measured in a spectrophotometer (name of spectrophotometer) at 595 nm. Three measurements were made for each cuvette by opening and closing the lid and then these measurements were averaged to ensure accuracy. The final measurements were then put through an equation (note equation) in order to achieve the correct standard curve. The cuvettes were correctly disposed of and the samples were stored.

In a new 1.5 mL screw cap tube 15 uL of proteins stock and 15 uL of 2x Reducing Sample Buffer (confirm this is what we used) were added. The tubes were vortexed for around 1 second and then briefly centrifuge (around 10 seconds) in order to pool the liquid. These samples were then boiled for five minutes. While the samples were boiling, the agarose gel was set up and the loading buffer was added (figure out correct loading buffer). The wells were also washed out one by one with the loading buffer. Once the samples finished boiling, they were immediately centrifuged for 1 minute and then one by one added to the wells in the gel. The lid was placed on and the gel ran for 45 minutes at 150 volts.

After the gel ran for the total length, the Western Blot protocol began. Extremely carefully the membrane (check what type) was removed and began soaking in Tris-Glycine Transfer Buffer. Once completed the cassette was cracked and the wells of the gel were trimmed. The gel was then also soaked in the Tri-Glycine solution. After fifteen minutes of soaking the blotting apparatus was loaded. The apparatus was loaded in this order: Anode (+++), 2 soaked filter papers, membrane, gel, 2 soaked filter papers, and cathode (---). The sandwich was then rolled out to ensure there were no air bubbles within it. The blot was run for 30 minutes at 20 volts.

Once finished, the blotting apparatus was taken apart and the gel and membrane were removed and put into separate containers. The membrane was immediately put into blocking solution for thirty minutes, while the gel was put into some sort of blue liquid (WHAT WAS THE LIQUID). Both trays were put onto a rotating plate at around 1 rev/sec during the thirty minutes. After the time had elapsed the gel was washing with water, while the membrane was rinsed off twice. The membrane was then covered with 10 mL of Primary Antibody Solution (check what type) and put on a rotating plate to incubate overnight.

FIND OUT THE REST OF THE STEPS FROM THE DAY YOU MISSED

Results:

Discussion