Determination of RNA:DNA Ratio in Bovine Liver Tissue

Introduction:

Concentration determination is a valuable measurement in the biological sciences. In particular, nucleic acid concentration, although it may seem relatively small compared to the macroscopic world, is worth measuring. Nucleic acids are polymers composed of nucleotides, which in living organisms are based on one of two sugars, ribose or deoxyribose, yielding the terms ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) (Karp, 2013). However, isolating the nucleic acids from the rest of the cells is the first obstacle. In 1957 W.C. Schneider developed a method for the determination of nucleic acid inside cells (Bregman, 2002). First, the chilled tissue must be well mixed so there is disruption at the cellular level also known as homogenization. The specific type of homogenization used for this experiment was by physically shearing the chilled tissue sample with a chilled Waring Blender to reduce the sample size (Burden, 2008). Next, the desired nucleic acids must be extracted from the homogenized sample using trichloroacetic acid (TCA) and ethanol. Cold TCA precipitates the nucleic acids, proteins and lipids from the rest of the homogenized sample. This precipitate containing the desired nucleic acids can be separated from the acid soluble supernatant by centrifugation in which the pellet contains the unpurified desired product (Bregman, 2002). For purification, ethanol which is a polar solvent is used to dissolve the polar fats. Finally, Schneider's method requires the use of hot TCA to dissolve out and partially degrade the DNA and RNA into solution.

Once the nucleic acid containing solution is obtained, specific nucleic acids, DNA or RNA can be colored so the compounds absorb light. Two colorimetric reactions were necessary for this experiment. The Diphenylamine reaction uses the diphenylamine reagent that contains acetic and sulfuric acids to cleave the DNA at some of the phosphodiester bonds and hydrolyze the glycosidic linkages between the sugars and purines when the DNA is heated. The sugars of the DNA, 2-Deoxyribose, are then converted to ω -hydroxylevulinyl aldehyde by the acid to react with the diphenylamine to produce the blue colored compounds that absorbs visible light. The intensity of the blue solution depends on the concentration of the DNA in solution; larger DNA concentrations correspond to more intense blue colored solutions. The other reaction uses orcinol reagent that contains hydrochloric acid to cleave some of the phophodiester bonds and hydrolyze the glycosidic linkages between the sugars and purines of RNA. The acid also converts sugars of the RNA, ribose, to furfural that can react with the orcinol in the presence of Iron (III) ions to produce green colored compounds in solution. The major difference between the two reactions is that the orcinol reaction colors all five membered sugar rings including the RNA rings and some of the DNA rings. 10% of the intensity of the green colored solution is caused by the DNA concentration (Bregman, 2002). The color reactions were also performed on known concentrations of pure DNA and pure RNA to set up a colorimetric assay in which there are solutions prepared with increasing concentrations.

Once isolated, the extracted, purified, and colored nucleic acid samples of DNA and RNA are prepared for a light absorption reading. The study of electromagnetic radiation emitted or absorbed by a given chemical species that can be used for quantitative analysis is known as spectroscopy (Zumdahl, 2010). Of the variety of spectroscopic techniques, this experiment used a spectrophotometer to measure the amount of visible light absorbed by the colored DNA and

RNA samples of nucleic acid extract from a bovine liver sample. The spectrophotometer used in this experiment is an instrument that spreads light from a tungsten lamp into its component wavelengths between 340 and 1000nm due to a diffraction grating. The sample absorbs wavelengths of light and the light that is not absorbed strikes the phototube, which converts the transmitted light energy to an electric current that is read by a meter (Bregman, 2002). To determine the concentration of the extract samples absorbance values were measured for known concentrations of pure DNA and pure RNA prepared solutions to be able to create a graph. This graph is known as a standard curve. The extracted sample absorption values could then be superimposed on this standard curve to determine the unknown DNA and RNA concentration is due to the Beer-Lambert law that states that absorbance is directly proportional to solute concentration and to the length of the light path (Bregman, 2002). **Procedure:**

Extraction of the Nucleic Acids

This experiment used 30g of frozen calf liver as the source of nucleic acids. This sample was homogenized by mixing the cubed sample in a chilled Waring Blender with 120mL of distilled water. Each lab group received 2.0mL of the liver homogenate.

5.0mL of cold 10% trichloroacetic acid (TCA) was added and mixed to the chilled homogenate. The solution was centrifuged at high speed for 2 minutes and the supernatant was drawn off using a Pasteur pipet. The pellet remaining in the centrifuge tube was resuspended in 5.0mL of chilled 10% TCA and spun in the centrifuge for another 2 minutes. Again the supernatant was drawn off and discarded. Then 10mL of 95% ethanol was added to resuspend the pellet. The sample was centrifuged for 2 minutes, and the supernatant was decanted to obtain the desired pellet. This ethanol wash was performed twice for further purification. 5.0mL of 5% TCA was added to the tube to disperse the purified pellet and the tube was placed in a 90°C water bath for 15 minutes during which the tube was agitated three times. After the warm water bath, the tube was centrifuged for 2 minutes and the desired supernatant was decanted into a test tube. A final wash of the pellet with 5.0mL of 5% TCA was done to ensure all of the nucleic acids were decanted into the test tube.

Diphenylamine Reaction

To perform the colorimetric assay a diphenylamine reaction was done with the nucleic acid extract. The preparation of the five-sample assay required six test tubes to be set up as show in the table below.

Tube	[DNA]	Volume (mL)	Nucleic acid	5% TCA	Diphenylamine
	$(\mu g/mL)$ in	of each DNA	extract (mL)	(mL)	reagent (mL)
	each standard	stock solution			
Blank	0	-	-	2.0	4.0
1	100	2.0	-	-	4.0
2	200	2.0	-	-	4.0
3	300	2.0	-	-	4.0
4	400	2.0	-	-	4.0
5	-	-	2.0	-	4.0

After all six tubes received the diphenylamine reagent dispensed from a buret, the tubes were inverted to mix the solutions. The tubes were placed in a boiling water bath for 10 minutes, cooled quickly, and then transferred to six cuvettes. The spectrophotometer was set to a wavelength of 600nm, the blank cuvette was put in the sample holder and the absorbance was adjusted to zero/100% Transmittance. The absorbance values of tubes 1-5 were measured and recorded as shown below.

Tube	$[DNA](\mu g/mL)$	A_{600}
1	100	0.193
2	200	0.355

3	300	0.524
4	400	0.648
5 (nucleic acid extract)	unknown	0.123

Orcinol Reaction

To determine the unknown RNA concentration a separate colorimetric assay was done using the orcinol reaction. Again, six tubes were set up according to the table below.

Tube	[RNA]	Volume (mL)	Nucleic acid	5% TCA	Orcinol
	(µg/mL) in	of each RNA	extract (mL)	(mL)	reagent (mL)
	each standard	stock solution			
Blank	0	-	-	3.0	3.0
1	100	0.4	-	2.6	3.0
2	200	0.4	-	2.6	3.0
3	300	0.4	-	2.6	3.0
4	400	0.4	-	2.6	3.0
5	-	-	0.4	2.6	3.0

Once all tubes received 3.0mL of orcinol reagent dispensed from a buret, the tubes were inverted to mix. The tubes were placed in a boiling water bath for 20 minutes, cooled quickly, and transferred to six cuvettes. The wavelength was set to 660nm on the spectrophotometer, and the blank cuvette was placed in the sample holder so the absorbance was set to read A = 0 and 100% Transmittance. The absorbance values of tubes 1-5 were measured and recorded and shown below.

Tube	[RNA] (µg/mL)	A_{660}
1	100	0.126
2	200	0.263
3	300	0.382
4	400	0.514
5 (nucleic acid extract)	unknown	0.182

Results:

Measuring and recording the absorbance values for both colorimetric assays allowed the data to be organized and analyzed. Since all the stock solution concentrations that were added to tubes 1-4 were known and the corresponding absorbance values were measured a standard curve was created to visualize the data. The standard curve plots absorbance versus known concentrations of the pure substance because the Beer-Lambert law states that absorbance is directly proportional to solute concentration and to the length of the light path, which in this case was held constant (Bregman, 2002).

The standard curve for the diphenylamine reaction using the known DNA stock solutions is shown below. This curve abides by the Beer-Lambert law and displays a direct relationship between the concentration and absorbance. As the concentration of the DNA increased the absorbance value increased, resulting in a positively sloped graph where the slope equaled $1.7 \times 10^{-3} \text{cm}^2/\mu g$, which represents the molar absorption coefficient (α) in the Beer-Lambert law. An important note is that on this plot the point of zero DNA concentration and zero absorbance, the blank cuvette's DNA concentration and absorbance, was graphed. The line on the graph is a line of best fit starting at the origin. From this graph it was found that the concentration of the previously unknown DNA extract was 72.35µg/mL. To determine this value the slope equation on the graph was used to calculate the concentration at an absorbance of 0.123. Graphically to find the concentration the absorbance value of 0.123 was located on the graph and then moving horizontally from that point until the line of best fit was hit. From the line of best fit, a vertical line downward was followed to the DNA concentration axis to read the extracted DNA concentration. Using the mass and volume from the homogenization of 30g bovine liver and 120mL distilled water the concentration of DNA in the original bovine liver was calculated to be 0.29µg of DNA per milligram of bovine liver.



The standard curve for the orcinol reaction that colored the RNA is also shown below. This positively sloped data plot of absorbance versus the known RNA concentrations also supports the direct relationship of these two variables of the Beer-Lambert law. The molar absorbance coefficient for the orcinol reaction was 1.3×10^{-3} cm²/µg. On this graph, too, the point of zero absorbance and zero RNA concentration was plotted and a line of best fit was produced to go through the origin. The process of determining the unknown RNA concentration of the nucleic acid extract for the orcinol reaction graph was different from the Diphenylamine reaction standard curve. The reason being is that there is a slight color contribution from the DNA concentration of the extract that was measured in the unknown "RNA" absorbance value (Bregman, 2002). Ten percent of the recorded absorbance of the unknown extracted RNA concentration. Thus, to

determine the absorbance of just the unknown RNA from the extract, 10% of 0.097, the absorbance at the concentration of DNA in the extract, was subtracted from the nucleic acid extract absorbance value 0.182 (absorbance value from the orcinol reaction). This computed the unknown RNA absorbance to be 0.172. Using the slope equation on the graph the unknown RNA concentration was found to be 132.54µg/mL. The ratio of RNA concentration to DNA concentration from the extract was 1.83. Initially the RNA concentration was 0.53µg of RNA per milligram of bovine liver.



Discussion

One of the simplest and most widely used methods to determine the amount of protein or nucleic acid present in a given solution is to measure the amount of light of a specific wavelength that is absorbed by that solution (Karp, 2013). A spectrophotometer was the instrument used during this experiment to measure the absorbance values of concentrated, colored solutions of nucleic acids

from a bovine liver sample. However, in order to measure the amount of light absorbed by DNA and RNA extracts the nucleic acids were isolated and purified from the rest of the liver tissue. Using known concentrated stock solutions of DNA and RNA the unknown concentrations of the extract were determined by colorimetry, a procedure in which the concentration of a compound is measured by the intensity of the color produced when the appropriate color reagents were added (Bregman, 2002). The blank cuvette used as a part of the colorimetric assay served to adjust the spectrophotometer prior to absorbance readings. This blank cuvette contained all of the same substances in the sample except the nucleic acids in order to set the instrument to compensate for the absorbance of the solvent. Therefore, the absorbance values recorded are indeed the amount of light the nucleic acids in solution absorbed.

The results from the Diphenylamine and Orcinol reactions that were organized into the standard curves above support the Beer-Lambert law. The direct relationship between the absorbance values and the concentration of nucleic acids resulted in a positive sloped plot because with more moles of nucleic acids in the 6mL of sample solution the more photons of light the sample could absorb. This result corresponds to a previous experiment done in the Cellular Biology laboratory at SUNY Cortland in which an absorption spectrum was created of different concentrations of erythrosine (Parsons and Schapiro, 1975). The higher concentrated sample had an overall larger curve than the less concentrated sample especially at the absorption maximum, the wavelength at which the absorption spectrum exhibits a peak (Bregman, 2002).

The unknown DNA extract concentration value that was determined, 72.35µg/mL and the absorbance value of 0.123 qualitatively correlated well with the other known DNA stock concentrations and those absorbance values. When preparing the colorimetric assay it was noted

that the blue color intensity observably increased as the concentration of the DNA increased and the unknown DNA concentration sample color was the least intense blue color.

The unknown RNA extract concentration value, which was greater than the DNA concentration of the extract, was 132.54µg/mL. The ratio between this RNA and DNA is 1.82, indicating that RNA has a higher concentration in bovine liver tissue cells than DNA concentration. These results are consistent with other experimental results regarding the concentration of nucleic acids in liver tissue. Musafa and Mittal performed a similar experiment in 1981 that measured the weights of proteins and the concentrations of RNA and DNA in the livers and brains of catfish to observe how nutrition influenced these measurements. In fact this lab used the same RNA extraction technique of Schneider. It was found that with normal feeding the concentration of RNA in the liver was 437.4µg/100mg, the concentration of DNA was 93.8µg/100mg, and the protein concentration was 17.6mg/100mg. This produced a 4.660 RNA/DNA ratio in the liver. In the brain the concentration of RNA was 113.8µg/100mg, the concentration of DNA was $44.5\mu g/100mg$, and the protein concentration was 8.8mg/100mg. This ratio in the brain of RNA/DNA was 2.552. One key point made was that high RNA/DNA ratios correspond with actively synthesizing and accumulating protein individuals (Musafa and Mittal, 1982). Thus, the liver is more involved in protein synthesis than other tissues of a body such as the brain. This explains the high ratio because it is the RNA strands of nucleic acids (mRNA, rRNA, and tRNA) that are more directly involved in protein synthesis (Karp, 2013).

A corresponding experiment that would be a logical follow up to the bovine liver experiment would be one in which other tissues of a calf were tested. It would be interesting to extract, purify, color and create standard curves of absorbance versus concentration for other tissues of the body. One could test where RNA concentration was larger or smaller and therefore where protein synthesis was more increased or decreased. Also, in these future experiments it would be advantageous to measure the protein concentration as well just at Musafa and Mittal had done. With a protein concentration one could directly make the connection that where there is a higher RNA concentration there is a higher protein concentration because RNA is involved in synthesizing proteins.

Nucleic acids are one of the four macromolecules of biology that hold the genetic information that allow cells to function (Sternfeld, 2015). The concentrations of DNA and RNA that make up nucleic acids do vary from tissue to tissue to allow cells to function properly and efficiently.

Works Cited

- Bregman, Allyn A. Laboratory Investigations in Cell and Molecular Biology. New York: John Wiley & Sons, Inc., 2002. Print.
- Burden, David W. "Guide to the Homogenization of Biological Samples." *Random Primers* 7 (2008): 1-14. Web.
- Karp, Gerald. *Cell and Molecular Biology* New York: John Wiley & Sons, Inc., 2013. Print.
- Musafa and Archanna Mittal. "Protein, RNA and DNA Levels in Liver and Brain of Starved Catfish Clarias batrachus." *Japanese Journal of Ichthyology* 28.4 (1982): 396-400. Web.

Parsons and Schapiro. Exercises in Cell Biology. McGraw-Hill (1975).

Sternfeld, John. "Nucleic Acids Lecture." SUNY Cortland. Sperry Hall, Cortland, NY. 6 March 2015. Lecture.

Zumdahl, Steven S. Chemistry Belmont: Brooks Cole. 2012. Print.