Introduction:

Despite anaerobic respiration being portrayed as the lower efficiency method of energy production in comparison to its aerobic counterpart, it still plays a crucial role in our physiology. LDH is one enzyme recently revealed to facilitate the pathway of lactic acid production by catalyzing a reaction between pyruvate and lactate, as shown by the reaction below.

$$\text{Pyruvate} + \text{NADH} \leftrightarrow \text{Lactate} + \text{NAD}^+$$

The purification, analysis, and understanding of LDH are essential to human health care. Presence of many diseases, such as cancer, elevates LDH in the blood, which can be detected. Cancer also is heavily reliant on LDH to build lactate, and can be attacked by inhibition of denaturation of LDH enzymes (Fiume, 2014).

Multiple purification steps were used to separate LDH from other proteins found within a cell. Firstly, because we understand that lactic acid formation occurs within the cytoplasm, we can safely conclude that LDH is also in the same area. Centrifuging will narrow down the proteins, eliminating proteins from the nucleus and other proteins residing in organelles.

Next, we used our knowledge of LDH in terms of its solubility. LDH is has a specific hydrophilicity can be easily precipitated out using ammonium sulfate precipitation. This process served to filter out other proteins that are either more or less soluble. At the end, a sample of proteins that are of similar solubility to LDH can be obtained.

LDH's affinity was also a tool that was utilized to further filter out other proteins via affinity chromatography. Since LDH has a high affinity towards NAD+, we can use this property to purify it. Nucleotide cofactors (NAD^+ in this case) are utilized by LDH, so Cibacron Blue-sepharose is an excellent affinity resin for this purification step (Markert, 1984).

Size is also a great factor to further purify the protein via size exclusion chromatography. LDH has a molecular weight of 146.6 kDa. Using a G100 grade of sephadex, which has a fractionation range of 4 to 150 kDa, we can purify LDH by eluding it right after the void volume.

Isoelectric points of LDH isozymes differ from one another. Isozymes that exist in the heart contain LDH that is mostly composed of H subunits, while other organs such as the muscle contain LDH composed of the M subunits (Shaw *et al*, 1963). Other organs have a different combination of the two subunits, and this property can help identify where the LDH came from via isoelectric focusing (Slattery *et al*, 1983), since each subunit has a different isoelectric point. By running the unknown tissue sample's LDH against standards in a gel, we can determine what the unknown tissue sample is.

The purification and identification of LDH subunits is important to further understand metabolism patterns in multicellular life. It is indeed an essential component in human biochemical processes, and understanding its properties can further the development of diseases and medicine for human civilization.

Materials and Method

It is critical to keep the samples in cold temperatures during the entire duration of the experiment. Low temperatures will cause proteases to denature and thus will be unable to digest LDH. This is especially critical before the sample has been purified.

Clarified Homogenate Centrifugation

The initial steps involved obtaining an unknown sample that contained an isoform of LDH and centrifuging at 20,000 xg for 10 minutes. This filtered out a clarified homogenate (CH) after discarding the pellet, which is a purification intermediate.

65% Ammonium Sulfate Cut

Next, a 40% ammonium sulfate cut was performed. Ammonium sulfate was added until 40% saturation was obtained, which involved adding 0.242g of ammonium sulfate per ml of sample. The sample was then centrifuged at 15,000 xg for 15 minutes and the pellet was discarded. A 65% cut was then performed by adding an additional 0.166g of ammonium sulfate per ml. discarding the supernatant

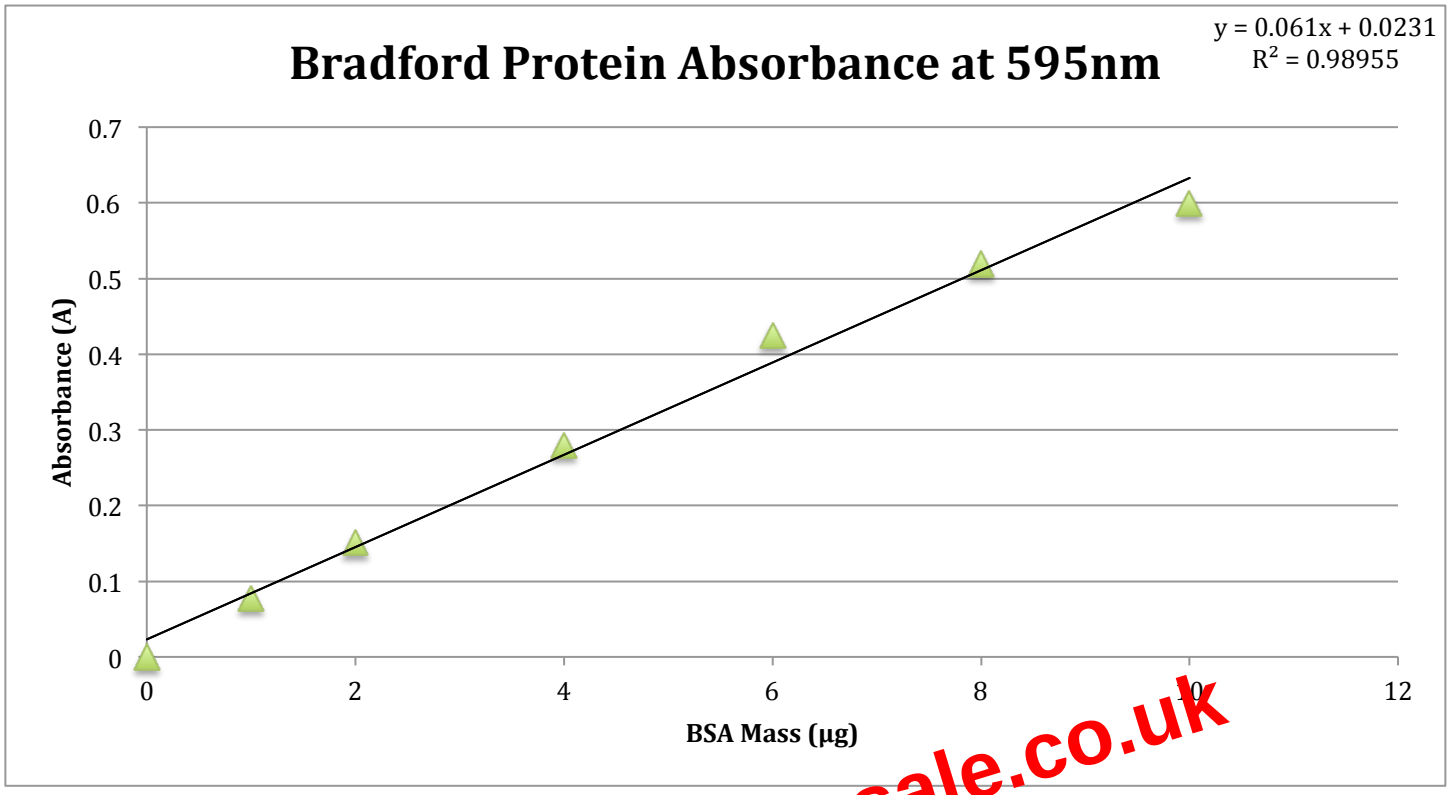

Fig. 6. Bradford Protein Absorbance Standard

The graph shows the Bradford protein absorbance at 595nm for different masses of BSA protein. Using this, we are able to estimate the amount of protein we have in our 4 samples. In order to ensure this works, we measured both 2ul and 10ul of each sample, so that at least one falls into the range of our BSA absorption values.

Below is a sample calculation for the CH sample.

$$y = 0.061x + 0.0231$$
$$0.109 = 0.061x + 0.0231$$
$$x = 1.4082$$

So in our CH sample, there was 1.4082µg of protein mass per 2µL of sample.

$$Protein\ Conc. = 1.4082\mu g \div 2\mu l$$
$$= 0.7041\frac{mg}{ml}$$
$$Act. Protein\ Conc. = 0.7041\frac{mg}{ml} * 10DF$$
$$= 7.041\frac{mg}{ml}$$

$$Total\ Protein = 7.041\frac{mg}{ml} * 87ml$$
$$= 612.567mg$$

Sample	Act. Protein Concentration (mg/ml)	Total Protein (mg)
CH	7.041	612.5670
65%	47.689	262.2895
Affinity	108.975	82.7120
Size Exclusion	51.190	61.4250

Fig. 7. Table containing calculated results for actual protein concentration and total protein for all purification intermediates.

Specific Activity Calculation Example (CH):

$$= Total\ Activity \div Total\ Protein$$
$$= 5.79U \div 612.5670mg$$
$$= 0.8223\frac{U}{mg}$$