Bradford method for determining unknown protein concentration biology essay

Science, Biology

BCM 254/256 Report assignment 1Bradford method for determining unknown protein concentrationAlandie

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Introduction:

1. 1 Aim:

The aim of the experiment is to determine the unknown concentration of a protein by using the Bradford method for protein quantification.

1. 2 Theory and principles:

Many different methods of determining proteins are used as all of them depend on different properties of amino acids. 1The Bradford method is a fast and fairly accurate method of determining the concentration of an unknown protein, 2 but it is influenced by the purity of the protein. The method uses a dye called Coomassie Brilliant Blue G250 (CBBG). The dye reacts with amino acids that carry positive charges and to a limited extent with aromatic amino acids to produce a blue colour. The side chain of the amino acid also determines the extent of the reaction with the CBBG, because each side chain has a different pKa at which it protonates in order to carry the positive charges. 3The protein used to perform this experiment

is bovine serum albumin (BSA). This protein has been used throughout the years so it is an effective protein to demonstrate the Bradford method. 4

1. 3 Application of Principles:

The Bradford method is recommended for use when determining the protein content of fractured cells or when accessing the concentrations for electrophoresis. 1 The reason for using the Bradford method in contrast to for example the Biuret method, is that the Bradford method is much faster and therefore you get results much faster than having to wait for a reaction to occur. 5Basic amino acids carry a large positive charge in an acidic pH, so there is an electrostatic interaction with the amino acids and the SO3- groups of the dye. This reaction stabilises the anionic form of the dye along with hydrophobic reactions, to result in higher absorption of the dye from 465nm to 595nm. This absorbance is directly related to the concentration of protein in the solution. This method for protein determination manipulates surface charges and the hydrophobic character of the solvent, to convert the protein structure into random coils. We assume that all the proteins will coil randomly in more or less the same degree. These coils are what allow the different concentrations of the protein to shine through the spectrophotometer through different absorbencies. When the protein binds to the SO3- group of the dye, the pKa value shifts causing the dye to turn blue. A standard protein curve is made to show the relationship of the concentration of the protein to that of the absorbency at 595nm. Through this curve you can determine the unknown protein concentration by use of Beer’s Law. 6

Experimental:

2. 1 List of reagents:

Stock of Bradford reagentBovine Serum Albumin (BSA) solutionPrepared protein A in the range of 0. 1-0. 8mg/ml

2. 2 List of apparatus: 2

1. Light spectrophotometer with maximum transmission in the range of 595nm. 2. 6 labelled test tubes

2. 3 Procedure: 3

1. Mix the Bradford reagent as follows: 100mg of Coomassie Brilliant Blue G250 dissolved in a mixture of 100ml 85% phosphoric acid, 50ml of 90% ethanol and 50ml of 1M NaOH. This is stored at 4˚C until a precipitation occurs, then discard. 2. Mix 0. 2g of the BSA with 20ml distilled water to prepare. That is 10mg/ml of protein solution. 3. Make a working Bradford reagent by mixing 10ml of the stock reagent with 250ml distilled water. 4. Prepare the test tubes as follows: BlankTube 1Tube 2Tube 3Tube 4UnknownDistilled water0. 1ml0. 1mg/ml protein0. 1ml0. 2mg/ml protein0. 1ml0. 4mg/ml protein0. 1ml0. 8mg/ml protein0. 1mlUnknown solution0. 1mlWorking Bradford4ml4ml4ml4ml4ml4ml5. After each addition of the working reagent, mix the resulting solution thoroughly. 6. Let the solution rest for 5minutes at room temperature. 7. Read the absorbance from the light spectrophotometer at 595nm for all the tubes as well as the blank.

Data and Calculations:

3. 1 Results:

Table of the absorbance at 595nm obtained from the different protein standards:

Protein Concentration (mM)

Absorbancy (595nm)

0. 00 (blank)0. 000. 100. 150. 200. 250. 400. 500. 800. 95Unknown0. 35The colour of each protein changes to a slightly different shade of blue, depending on the level of reaction with the Coomassie Brilliant Blue G250.

3. 2 Calculations with statistical manipulation:

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The method used to determine the concentration of the unknown protein is by using Beer's Law: A= Ɛ x c x l (Where l = 1)Ɛ is the line gradient and can be determined by the methodm = (Y2 – Y1) / (X2 – X1)The 2 data points used are: (0; 0) and (0. 50; 0. 60)So the gradient of the line (Ɛ) = (0. 60 – 0) / (0. 50 – 0)= 1. 2Thus 0. 35 = 1. 2 x c (c = concentration)c = 0. 29mMAnother way to determine the unknown concentration of the protein is to read the absorbency of the unknown protein from the graph to the specific protein concentration. That makes the concentration of the unknown protein around 0. 29mM.

Results and discussion

From the known concentrations of proteins it is easy to determine the unknown concentration of the same protein by using Beer's Law as was demonstrated in the results. We can also read the unknown concentration directly from the standard curve although it is not as accurate as using Beer's law. With this method the unknown concentration has been calculated as 0. 29mM.

Conclusion:

The aim to determine the unknown concentration of the protein has been achieved by using the Bradford method. It is an easy and quick method for determining the absorbency of a protein with an unknown concentration and in conjunction with Beer's law it is easy to determine the concentration.