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Assignment

1. What do you understand by the term “biological value of protein”
2. List and explain the various methods of assessment of protein quality.

Biological value of protein is a measure of the proportion of absorbed protein from a food which becomes incorporated into the proteins of the organism’s body. It captures how readily the digested protein can be used in protein synthesis in the cells of the organism. Proteins are the major source of nitrogen in food. Biological value of protein assumes protein is the only source of nitrogen and measures the proportion of this nitrogen absorbed by the body which is then excreted. The remainder must have been incorporated into the protein of the organism’s body. A ratio of nitrogen incorporated into the body over nitrogen absorbed gives a measure of protein usability.

For accurate determination of biological value of protein

1. The test organism must only consume the protein or mixture of proteins of interest
2. The test diet must contain no non-protein sources of nitrogen.
3. The test diet must be of suitable content and quality to avoid use of the protein primarily as an energy source.

Factors that affect biological value of protein

The determination of biological value is carefully designed to accurately measure some aspects of protein usage whilst eliminating variation from other aspects. When using the test care must be taken to ensure the variable of interest is quantified by biological value. Factors which affect biological value of protein can be grouped into prosperities of the protein source and properties of the species or individual consuming the protein.

Properties of the protein source

1. Amino acid composition, and the limiting amino acid, which is usually lysine
2. Preparation/ cooking
3. Vitamin and mineral content

Amino acid composition is the principal effect. All proteins are made up of the 21 biological amino acids.

Properties of the test species or individual

1. Under test conditions
2. In everyday life

Factors with no effect

Biological value of protein is designed to ignore variation in digestibility of a food which in turn largely depends on the food preparation.

Common foodstuffs and their values

1. Whey protein- 96
2. Whole soy bean- 96
3. Human milk- 95
4. Chicken egg- 94
5. Soybean milk- 91
6. Cow milk- 90
7. Cheese- 84
8. Fish- 76
9. Beef- 74.3
10. Whole wheat- 64

2

Various methods of assessment of protein quality

1. General Quantification: UV-Vis, Bradford, and Activity Assays:

Measuring the concentration of a protein sample is generally required for many of the methods outlined later in this article to be effective.

While UV-Vis spectrophotometry and Bradford assays are high-throughput and are used in almost every biochemistry lab, they are relatively crude compared to enzymatic activity assays. This is because UV-Vis and Bradford assays results depend on the *total* protein within a sample, not just your protein of interest. In contrast, activity assays are target specific and have the additional benefit of measuring the fraction of *active* protein in a purified sample. However, not all proteins can be quantified with an activity assay.

1. Size Analysis: Electrophoresis (Native/Denaturing PAGE):

Like the quantification methods described above, electrophoresis is widely employed by biochemists and can provide a general picture of both the size of your target protein and whether there are other protein-based impurities present. However, you’ll want to get an approximation of how concentrated your protein is before performing electrophoresis—lest you risk having to repeat running a gel at 6:30pm on a Friday night!

There are several types of electrophoresis methods, the most common being [denaturing SDS-PAGE](https://bitesizebio.com/580/how-sds-page-works/). Samples are first denatured with SDS (a detergent) then separated by mass on a polyacrylamide gel matrix using an electric field. In native PAGE, protein separation is more complex and is based on net charge, size, *and* shape of the native structure. In both techniques, you can identify if degradation is present in a sample by a ‘smeared’ band- but this can also occur if you have overloaded the gel with too much protein.

Some challenges of electrophoresis include that it does not reveal low-level impurities or minute size differences. Electrophoresis also requires samples with a concentration between 0.1 and 2 mg/mL to provide clear results.

1. Size Analysis: Mass Spectrometry:

Mass spectrometry is a very powerful analytical technique that can identify post-translational modifications with great accuracy and precision, which are not easily visualized with the techniques described above. Ionizing mass spectrometry works by separating proteins or peptides by mass and charge, accelerating them onto a detector, and creating a unique spectrum for each protein (or protein fragment).

The drawbacks of relying solely on mass spectrometry for assessing protein quality are that it is relatively low-throughput and requires extensive sample preparation. In addition, it is difficult to assess whether proteins in a sample are intact, since the process is denaturing and does not identify misfolding events.

1. Homogeneity: Dynamic Light Scattering-

If your protein appears pure using the methods above, it’s time to check to ensure that it is dispersed within the sample (i.e., not aggregating). Dynamic light scattering (DLS) uses polarized laser light to measure the level of diffraction in a sample with small molecules (or in our case, proteins!). The amount of scattering that occurs is an effect of the hydrodynamic radius of the particles in solution as the sample travels through the instrument.

While DLS is an easy-to-use technique that provides excellent qualitative information, it doesn’t provide a totally comprehensive picture of the size distribution in a protein sample since aggregates can easily overwhelm the detector. This technique is also not suitable for assessing quaternary structures (i.e., dimers versus monomers). With that said, the convenience of DLS combined with its ability to reveal aggregate formation over time makes it a widely used method for assessing homogeneity.

1. Microfluidic Diffusional Sizing (MDS)

Microfluidic diffusional sizing (MDS) as used in the Fluidity One system by Fluidic Analytics is a fast and simple option to measure protein size and concentration, which together give a good indicator of quality. MDS uses microfluidic chips to run the protein sample into a channel where it flows alongside an auxiliary fluid in a steady state laminar flow – with no mixing. The only way proteins can move from one stream to the other is by diffusion, which occurs at a rate proportional to their size (hydrodynamic radius, Rh) after some diffusion, the two streams are split again and the proteins labeled. The ratio of diffused and undiffused is used to calculate the Rh.

MDS avoids some pitfalls of other technologies. There is no interaction between the protein and a matrix, like in electrophoresis, and samples are run in their native state. The workflow required is simple with results under 10 minutes using samples with concentrations as low as ~10µg/mL