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Journal of Proteomics & Bioinformatics

Commentary

Recent Advances in the Field of Protein Sequencing

Mohibullah Shah*

Department of Biochemistry, Bahauddin Zakariya University, Multan-66000, Pakistan

DESCRIPTION

Determining amino acid composition

Prior to attempting to uncover the ordered sequence of a protein, it is frequently advantageous to know the protein's unordered amino acid composition, as this information can be used to identify faults in the sequencing process or discriminate between ambiguous results. Knowing the frequency of different amino acids can help you choose the right protease for the job.

Hydrolysis: A sample of the protein is hydrolyzed by heating it to 100°C–110°C for 24 hours or longer in 6 M hydrochloric acid. Proteins containing a lot of bulky hydrophobic groups may need to be heated for longer. These circumstances, however, are so intense that some amino acids are destroyed. Biochemistry Online (BO) recommends heating different samples for varied amounts of time, analysing each resulting solution, and extrapolating back to zero hydrolysis time. This cannot rule out all possible false-positive interactions, e.g., those resulting from high-abundance proteins Mellacheruvu, et al. [1] and protein-DNA interactions.

Separation and quantitation: Ion-exchange chromatography can be used to separate the amino acids, which can then be derivatized to make them easier to detect. The amino acids are usually derivatized before being resolved using reversed phase HPLC. The NTRC uses sulfonated polystyrene as a matrix, adds amino acids in acid solution, and passes a buffer of steadily increasing pH across the column as an example of ion-exchange chromatography. When the pH hits their isoelectric points, amino acids are eluted. Following the separation of the amino acids, the amounts of each are measured by adding a reagent that produces a coloured derivative. If the amount of amino acids is greater than 10 nmol, ninhydrin can be employed; when combined with proline, it produces a yellow colour, and when reacted with additional amino acids, it produces a vibrant purple colour. The absorbance of the resultant solution is related to the concentration of amino acid Alonso-Lopez, et al. [2]. Fluorescent derivatives can be made utilising reagents like ortho-phthaldehyde (OPA) or fluorescamine in very small amounts, as low as 10 pmol. The Edman reagent can be used to make a UV-detectable derivative during pre-column derivatization.

N-terminal amino acid analysis: There are a variety of chemicals available for labelling terminal amino acids. They all react with amine groups and will thus bind to amine groups on the side chains of amino acids like lysine; as a result, when interpreting chromatograms, care must be taken to ensure that the correct area is selected. Sanger's reagent (1-fluoro-2, 4-dinitrobenzene) and dansyl derivatives like dansyl chloride are two of the most commonly used reagents. The same issues apply as for determining amino acid composition, with the exception that there is no need for a stain because the reagents yield coloured derivatives and only qualitative analysis is necessary.

CONCLUSION

Proteins are made in biology by translating messenger RNA (mRNA), with the protein sequence derived from the mRNA codon sequence. The mRNA is created by the transcription of genes and can be changed further. Hence, the complete amino-acid sequence of the protein was subsequently deduced using the sequence of the cloned DNA.

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Corresponding to: Mohibullah Shah, Department of Biochemistry, Bahauddin Zakariya University, Multan-66000, Pakistan, E-mail: mohibusb@12gmail.com

Received: Aug 06, 2021;**Accepted:** Aug 20, 2021;**Published**: Aug 27, 2021

Citation: Shah M (2021) Recent Advances in the Field of Protein Sequencing. J Proteomics Bioinform.14:549.