Opinion

Type of stains used in detection of protein in gel

Volume 5 Issue 4 - 2017

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Received: March 30, 2017 | **Published:** April 12, 2017

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Proteomics a rapidly developing field, for decades, polyacrylamide gel electrophoresis and related blotting techniques have formed the core technologies for protein analysis. And the choice of staining techniques depends on the availability of imaging equipment in the lab in many cases.

Protein separation by two-dimensional electrophoresis (2DE) is largely used in proteomic approaches because of both high resolution and the availability of powerful image analysis software for gel comparison and compatibility with subsequent protein characterization by mass spectrometry.[1] For these various aspects, the selection of the protein staining procedure is of major importance.[2] Based on two independent biochemical characteristics of proteins, 2DE combines isoelectric focusing, which separates proteins according to their isoelectric point, and SDS-PAGE, which separates them further according to their molecular mass . The next typical steps of the flow of gel-based proteomics are spots visualization and evaluation, expression analysis, and finally protein identification by mass spectrometry. In order to take advantage of the high resolution capacity of 2DE, proteins have to be completely denatured, disaggregated, reduced and solubilized to disrupt molecular interactions and to ensure that each spot represents an individual polypeptide. Proteins can be stained before the open access Materials (pre-electrophoretic protein stain), or after 2DE separation (post-electrophoretic protein stain). Classically, Coomassie blue was the most widely used non-covalent dye for post-electrophoretic protein staining.[3] However, it suffers from a low sensitivity in protein detection, including in the improved colloidal version.[4] In contrast, the other classical protein stain, silver nitrate, displays an excellent sensitivity but could interfere with protein analysis by mass spectrometry.[5] In the last decade, different fluorescent dyes have been introduced.[6] These encompass Sypro Ruby,[7] and Ruthenium red-based dyes.[8] However, their present use remains relatively limited, probably due to their cost and/or technical difficulties.

luminescent Sypro protein gel stains are revolutionizing the detection of the total-protein. Sypro protein gel stains exhibit several important characteristics that together make them far superior to traditional staining methods,[9] including:

i. Fast and easy staining protocols.

ii. High sensitivity.

iii. Minimal protein-to-protein variation in staining.

iv. Broad linear quantitation range.

v. Compatibility with subsequent microanalysis and a variety of instrumentation.

Conventional methods for universal pro-ling of proteins in gels include Coomassie brilliant blue staining[10] and silver staining[11] Although Coomassie brilliant blue is an inexpensive reagent, its staining is relatively insensitive and time consuming. Silver staining may be up to 100times more sensitive than Coomassie brilliant blue staining, but it is relatively expensive and entails several labor-intensive and time sensitive steps. Silver staining also exhibits a high degree of protein to protein variability; staining intensity and color are very dependent on each polypeptide's sequence and degree of glycosylation, and some proteins are detectable only as negatively stained patches. Moreover, silver staining shows very poor linearity with protein concentration and poor reproducibility in staining from gel to gel, making it inadequate for comparative studies of protein expression in cells. During the last decade, different fluorescent dyes were introduced and proved to combine high sensitivity and compatibility with mass spectrometry. These encompass both commercially available stains, such as the series of Sypro,[12] and Ruthenium red-based dyes for which synthesis procedures were published. Sypro Ruby was described to combine sensitivity close to that of silver staining, and the good properties of classical organic stains such as Coomassie blue. Sypro Ruby is a luminescent ruthenium complex that interacts non-covalently with proteins thanks to a mechanism similar to the one of the colloidal Coomassie blue stain. As no irreversible modification of amino acids was operated during staining, satisfactory mass spectrometry compatibility was expected.[12] Sypro Ruby allowed stable sequence coverage regardless of spot intensity with a capacity of spots identification near the one of the colloidal Coomassie blue.[13] A constant identification of protein was shown independently of protein quantity. Additionally, Sypro Ruby was previously showed to have a broader linear dynamic range and a higher sensitivity than silver nitrate,[14,15] suggesting a profitable use of this dye for large scale proteomic analysis. Nevertheless, the necessity of a fluorescent scanner added to the cost of the dye itself has limited the use of Sypro Ruby.

Acknowledgements

None.

Conflict of interest

The author declares no conflict of interest.

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