AUCTORES
Globalize your Research

Journal of Surgical Case Reports and Images
Bon E.I *

Open Access

Review Article

Electrophoresis of Muscle Proteins

Bon E.I *., Maksimovich N.Ye., Kostyukevich D.S., Kalko

Grodno State Medical University.

***Corresponding Author:** Bon E.I, Grodno State Medical University.

Received Date: March 03, 2026; **Accepted Date:** March 19, 2026; **Published Date:** April 07, 2026

Citation: Bon E.I., Maksimovich N.Ye., Kostyukevich D.S., Kalko, (2026), Electrophoresis of Muscle Proteins, *J, Surgical Case Reports and Images*, 9(3); **DOI:10.31579/2690-1897/313**

Abstract

Electrophoresis of muscle proteins is a technology that allows us to look deep into muscle tissue and separate a complex mixture of proteins into individual components. This method is based on the movement of charged molecules in an electric field. Muscle tissue consists of several groups of proteins, each of which can be studied using electrophoresis: myofibril proteins, sarcoplasm proteins, and stroma proteins.

Keywords: proteins, electrophoresis, muscles

Introduction

Using free electrophoresis, more than fifteen separate components can be detected in muscle extracts, which differ in their isoelectric points and, therefore, have different mobilities in an electric field. A significant portion of these components has been identified with previously studied muscle proteins such as myosin, actomyosin, myogen fraction proteins, myoalbumin, and others. The electrophoretic pattern of protein extracts obtained from muscle tissue under specific experimental conditions also depends on the composition and concentration of the extracting saline solutions, the extraction time, and the objects of study. The article reveals the features of the fractional composition of muscle proteins in different types of musculatures under various functional conditions, changes in the fractional composition during philo- and ontogenesis, and in various forms of muscle pathology, etc. One of the disadvantages of free electrophoresis is the need to work with a significant amount of protein solution. When using Tiselius-Svensson devices, at least 10 ml of protein solution is required for each experiment, while Antweiler's device requires at least 1-2 ml of solution. Obtaining such a large amount of solution from micro-objects, such as insect muscles, single muscle fibers, or small groups of muscle fibers, is extremely challenging. This difficulty can be overcome using zone-specific microelectrophoresis, which requires a much smaller amount of sample.

Most protein analytical electrophoreses are achieved by separation in polyacrylamide gels under conditions that dissociate proteins into individual polypeptide subunits and minimize aggregation. Most commonly, the anionic detergent sodium dodecyl sulfate (SDS) is used in combination with a reducing agent (β-mercaptoethanol or dithiothreitol) and heating to dissociate proteins before loading into the gel. SDS binding denatures polypeptides and imparts a negative charge that masks their intrinsic charge. The amount of SDS bound is generally independent of sequence and proportional to molecular weight; at saturation, approximately one SDS molecule binds per two amino acids or ~1.4 g SDS per g polypeptide. Consequently, the migration of SDS-polypeptide complexes in an electric field is proportional to the relative size of the polypeptide chain, and its molecular weight can be estimated by comparison with protein markers of known molecular weight. However, hydrophobicity, highly charged sequences, and certain post-translational modifications, such as glycosylation or phosphorylation, can also affect migration. [1]

Ionic liquids (ILs), as non-molecular type solvents, have excellent physical and chemical properties, making them useful in important separation applications in gas chromatography, liquid chromatography, etc. capillary electrophoresis. Among the many potential applications of ionic liquids in separation science, capillary electrophoresis can utilize its enhanced resolution effect in the analysis of proteins and carbohydrates, through the formation of intermolecular interactions such as hydrophobic, hydrogen bonding, or electrostatic. IL and polymeric ionic liquids (PIL) are also excellent choices as background electrolyte (BGE) additives for capillary coatings in CE, which is especially important for protein analysis. Ionic liquids (IL) are liquefied salts with a melting point of <100 °C, although many ILs already liquefy at room temperature or below. ILs consist of anions and cations, representing a type of solvent with a non-molecular ionic character. The exceptional properties of ILs include low volatility, excellent thermal stability, unique electrochemical characteristics, adjustable viscosity, and compatibility with other solvents, making them useful in various analytical applications such as chromatography, capillary electrophoresis, mass spectrometry, and various sample pre-concentration methods. It is crucial to note that ILs can serve as an alternative to low-cost green solvents, replacing their flammable organic counterparts. Ionic liquids can be classified as highly polar solvents that can dissolve and stabilize dipole or charged solutes. The solubility properties of ILs are mainly influenced by the hydrogen donor/acceptor capacity of the salt and

the degree of charge localization on the anions. The formation of hydrogen bonds, as well as electrostatic, π–π, and ion–dipole interactions with the solute molecules, can affect the efficiency and selectivity of electrophoretic separations. The physicochemical properties of ILs can be precisely tuned by selecting the appropriate cation and anion for the specific separation problem, making them considered "designer solvents." The combination of different cation-anion pairs leads to a variety of ionic liquid options. As for anionic components, the high thermal stability of ILs, with a decomposition temperature typically above 400 °C, varies depending on the anions, which have a close relationship with their coordinating nature, nucleophilicity, and hydrophilicity. On the other hand, ionic liquids exhibit increased thermal stability with shorter chain lengths and more substituents in the cation. The viscosity of ILs is typically higher than that of water and decreases with increasing temperature. Their density primarily depends on the molar mass of the anion. The solubility of ionic liquids in water depends on the nature of the anion, temperature, and the length of the alkyl chain of the cation. Given these excellent physical and chemical properties of non-molecular solvents, this review article will focus on the application of ionic liquids in capillary electrophoresis, protein separation, and carbohydrate separation.[2]

Western blotting (immunoblotting) is a powerful and widely used technique that can detect or semi-quantitatively determine a single protein from complex mixtures of proteins extracted from cells or tissues. The automation of western blotting was achieved using the Protein Simple capillary electrophoresis system (San Jose, California). The original Protein Simple apparatus, Simon, used nanovolume capillaries to separate proteins through a stacking and resolving matrix. After gel electrophoresis, the proteins are immobilized by photo-initiated blotting and subsequent antibody incubations. Gel electrophoresis separates the proteins based on their size. Large proteins travel less on the gel than smear the proteins when an electric charge is passed through the gel. The protein lands on the positive electrode because the proteins are negatively charged (due to SDS binding). [3]

Proteins are an important class of macromolecules that play a crucial role in all living organisms. They provide structural support, promote growth, function in the immune system, and serve as catalysts, transport molecules, and storage compounds, among other applications. It is this versatility that allows us to use these functional units of life to understand the balance between healthy and diseased states at the molecular level. To study proteins, we must first be able to isolate and characterize them. There are many analytical methods for protein separation, with electrophoresis being one of the most well-studied and widely used. Although early work on electrophoresis, based on Faraday's laws of electrolysis, dates back to the nineteenth century, the growth of electrophoresis as a separation method is commonly attributed to Arne Tiselius, who successfully separated horse serum into albumin, α-, β-, and γ-globulin in the 1930s. This discovery, combined with Tiselius' work on adsorption and electrophoresis, led to his Nobel Prize in Chemistry in 1948. In the following decades, various methods of electrophoresis were developed, including zone electrophoresis, isoelectric focusing, and isothachophoresis. Notable at this time was the surge of gel electrophoresis as a separation method, which began with starch gels and in 1970 gave rise to what is now known as modern polyacrylamide gel electrophoresis with SDS (SDS-PAGE) . The use of tubes with a narrow inner diameter date back to the 1960s, when Stellan Hjerten demonstrated the use of a tube with a narrow inner diameter of 3 mm, which compensated for convective issues by rotating the tube with a narrow inner diameter. However, it was not until 1981, when Jorgenson and Lukacs developed the capillary zone electrophoresis technique, that CE began to gain traction. They used a tubular glass capillary with an internal diameter of 75 μm and a fluorescent detector on a column, which was subjected to a voltage of up to 30 kV, to demonstrate the separation of amines, amino acids, and dipeptides. The use of smaller capillaries resulted in more efficient heat dissipation due to the increased surface area to volume ratio. This allowed for higher voltages to be applied to the system, resulting in increased efficiency and reduced separation times. Later, it was shown that CE technology can use gels as a separation medium, leading to capillary gel electrophoresis methods. This work by Hjerten demonstrated separation in capillary tubes with an internal diameter of 0.05-0.30 mm using both agarose and polyacrylamide gels. Proteins present in quantities as low as 0.01 μg/mL were separated and detected using a capillary ultraviolet (UV) detection system. In the decades since these discoveries, CE and CE-SDS have undergone many advancements and have been applied in a wide range of fields.[4]

Electrophoresis of high-molecular-weight proteins (more than 500 kDa) of muscle myofibrils is difficult using traditional procedures. The mobility of these proteins was affected by the heating time in the sample buffer, the use of 2-mercaptoethanol in the top reservoir buffer, and the pH of the separating gel in the sodium dodecyl sulfate stacking gel system. Heating the samples for 4 minutes (compared to a shorter time), adding 2-mercaptoethanol to the upper tank buffer, and reducing the pH of the separating gel to 8.6 all increased the mobility and resolution of high-molecular-weight proteins on polyacrylamide gels. It was found that the sulfhydryl reducing agents commonly used in protein sample buffers (2-mercaptoethanol and dithiothreitol) migrate at the front of the electrophoretic dye. Incorporating 10 mM 2-mercaptoethanol into the top reservoir buffer or blocking free sulfhydryl groups with N-ethylmaleimide prevented the formation of intermolecular disulfide bonds during electrophoresis. Adding 10 mM 2-mercaptoethanol to the buffer used for electroblotting also increased the efficiency of protein transfer to nitrocellulose.[5]

The inclusion of methanol in the separating gel affected the electrophoretic mobility of proteins in a wide range of molecular weights. There was a greater separation of fast and slow isoforms of human MLC1, as well as a separation and high resolution of fast and slow isoforms of three isoforms of myosin heavy chain that are expressed in human skeletal muscles in the same gel format. Moreover, the same separating gel format significantly altered the electrophoretic mobility of at least one isoform of tropomyosin in human striated muscles. It is possible that the inclusion of methanol in SDS-PAGE gels can improve the separation of other proteins that are expressed in muscles, other tissues, and cell types.[6]

The sheep's latissimus dorsi muscle was electrically trained, which caused a rapid and slow transformation of fiber type. Using a combination of one - and two-dimensional gel electrophoresis methods with computer analysis, we analyzed the altered expression of contractile protein isoforms at the protein and mRNA levels during a temporary course of electro-training that lasted up to 5 months. Analysis of the myosin heavy chain and the regulatory myosin light chain showed predominant expression of their slow isoforms (86% and 92%, respectively) after 3 months of training. However, at the same time, analysis of tropomyosin showed that the slow isoform of the α subunit accounts for 64% of the total α subunit expression.The switching of troponin T isoforms was slower over the same period of time than that of tropomyosin and the thick filament proteins studied. Analysis of troponin T revealed 5 fast and 2 slow isoforms in sheep, of which the second slow isoform became clearly visible only after 5 months of training. At this point, the two slow isoforms were more prevalent than their fast counterparts. This suggests that there may be a wide heterogeneity of fast and slow isoform combinations in the thin filament of skeletal muscles.[7]

The application of two-dimensional gel electrophoresis has played a key role in the systematic identification and detailed characterization of the

protein components of skeletal muscles. The changes in protein during myogenesis, muscle maturation, fiber type specification, physiological adaptation of muscles, and natural aging of muscles have been thoroughly studied using O'Farrell's original method or slightly modified gel electrophoresis techniques. Over the past 40 years, the combined use of isoelectric focusing in the first dimension and sodium dodecyl sulfate gel electrophoresis with polyacrylamide plates in the second dimension has been successfully used in several hundred published studies on gel-based skeletal muscle biochemistry.[8]

Two-dimensional difference gel electrophoresis (2D-DIGE) is a method of electrophoresis in an acrylamide gel for the separation of proteins and quantitative determination in complex mixtures. This method overcomes some of the limitations of conventional two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) by offering improved sensitivity, more limited experimental variations, and precise alignment within the gel. 2D-DIGE is based on the direct labeling of proteins with isobaric fluorescent dyes (known as CyDyes: Cy2, Cy3, and Cy5) before isoelectric focusing (IEF).Here, up to two samples and a reference pool (internal standard) can be mixed and loaded into the IEF for the first measurement before SDS (sodium dodecyl sulfate)-PAGE separation in the second measurement. After electrophoretic analysis, the gel is sequentially visualized at a specific excitation wavelength for each dye and scanned individually. For each individual protein spot, the intensities recorded at different wavelengths are integrated, and the volume ratio is normalized relative to the internal standard. This allows for immediate assessment of changes in protein quantity under different testing conditions. In addition, proteins of interest can still be cut and identified using traditional mass spectrometric methods and further analyzed using other biochemical methods.[9]

The use of the main proteomics method for the visualization of proteins in meat compositions is two-dimensional electrophoresis (2DE), which allows for the parallel separation and analysis of hundreds and thousands of individual protein molecules. As a result of 2DE, "spotty" structures are formed, where each individual spot represents a specific protein. The intensity of the spot's staining indicates the quantitative content of protein fractions in the sample [10]

Solvent additives, including NaCl, arginine hydrochloride (ArgHCl), glycine, and sucrose, are used to increase protein stability or reduce protein aggregation. Since these additives are used at relatively high concentrations, we first confirmed that they do not interfere with native gel electrophoresis. Native agarose gel electrophoresis showed that heat-induced aggregation of bovine serum albumin (BSA) was slightly reduced by NaCl and ArgHCl. In contrast, glycine and sucrose had minor effects. ArgHCl and NaCl promoted the thermal aggregation of monoclonal antibodies (mAbs), whereas glycine and sucrose stabilized the native monoclonal antibody. Arginine methyl ester inhibited the thermal aggregation of lysozyme and, to a much lesser extent, BSA.[11]

Polyacrylamide gel electrophoresis plays an important role in the analysis of the function of muscle structural proteins. Electrophoretic studies have established the subunit structures of muscle proteins, characterized their multiple forms, and revealed changes in the composition of subunits or shifts in the distribution of isoforms of specific proteins during muscle development, stimulation, or denervation. Phosphorylation of proteins during muscle contraction is preferably studied using two-dimensional gel electrophoresis. The same method has demonstrated protein changes in human neuromuscular diseases.[12]

Talmadge and Roy (J. Apple. Physiol. 1993, 75, 2337–2340) previously established a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) protocol for separating all four isoforms of the heavy chain of skeletal muscle myosin (MHC) (MHC I, IIa, IIx, IIb); however, when applied to human muscles, the type II MHC isoforms (IIa, IIx) are not clearly distinguished. The MHC specificity of each band was confirmed by Western blotting using three monoclonal IgG (mAb) antibodies, immunoreactive against MHCI (mAb MHC, Novacastra Laboratories), MHCI+IIa (mAb BF-35) and MHCIIa+IIx (mAb SC-71).[13]

Standard experimental procedures for continuous polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate have been modified to provide more efficient separation and improved resolution of myofibrillar proteins. The system uses a working gel consisting of 10% acrylamide with 0.1% bisacrylamide cross-linking agent (100:1), including 400 mM Tris/glycine (pH 8.80), 0.1 mM ethylenediaminetetraacetate, 5% glycerol, and 0.1% sodium dodecyl sulfate.[14]

An electrophoretic method using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) has been developed, which provides high-resolution separation of the known heavy chains of rabbit skeletal muscle myosin with excellent reproducibility. The gel with a total length of 10 cm consists of (i) a first gel for laying with a total gel concentration of 3.5% (T) and a pH of 6.8, (ii) a first gel for separating 6.6%T and a pH of 8.8, (iii) a second gel for laying 6.6%T and a pH of 6.8, and (iv) a second gel to separate 8,8%T and pH 8,8. With this composition, the minigel system allows the separation of six isoforms of the myosin heavy chain (MHC) at room temperature without cooling and for 8 hours. [15]

When used in long (32 cm) separation gels, pulse-field electrophoresis not only significantly improves the resolution of MHC isotypes compared to conventional systems, but also reduces common artifacts associated with long running times, such as blurred bands and misalignment of closely spaced bands. In addition to the increased resolution of protein bands, pulse-field electrophoresis also allows the detection of bands corresponding to previously unidentified MHC isotypes in mammalian and avian tissues. For example, in rat myocardium, pulse-field electrophoresis revealed three bands of MHC isoforms, two of which appear to correspond to two subtypes of alpha-MHC. It is known that alternative splicing of the rat alpha-MHC gene results in the formation of two types of isoforms that differ in the inclusion (or exclusion) of a single glutamine residue, and their relative expression levels are well-correlated with the amounts of each band identified in this study.[16]

2D DIGE, two-dimensional differential gel electrophoresis, is a technology used to study protein expression on two-dimensional gels. Protein samples are labeled with different-colored fluorescent dyes designed to avoid affecting the relative migration of proteins during electrophoresis. [17]

The proteome of skeletal muscles consists of a large number of diverse protein species with a wide and dynamic range of concentrations. Since mature skeletal muscles are characterized by a specific combination of contractile cells with different physiological and biochemical properties, it is important to determine the specific differences in the protein composition of fast, slow, and hybrid fibers. Fluorescent two-dimensional gel electrophoresis (DIGE) is a powerful comparative tool for analyzing the differences between fast and slow muscles based on fiber type. [18]

Myofibrillar and sarcoplasmic proteins were extracted from pork meat (M. Longissimus dorsi) and then separated by capillary gel electrophoresis (CGE). Migration time and peak areas of individual protein molecules in the electropherogram were analysed. The electropherograms obtained after the separation of myofibrillar proteins contained 53 well-separated peaks, of which the following were identified: thymosin, myosin light chain-3 (MLC-3), myosin light chain-2 (MLC-2), troponin C, troponin I, myosin light chain-1 (MLC-1), tropomyosin 1, tropomyosin 2, troponin T, actin, desmin, troponin, C protein, and myosin heavy chain (MHC). The relative concentration of the identified myofibrillar proteins was 74.5%. Of the 56

separated sarcoplasmic proteins the following were identified: myoglobin, myokinase, triosephosphate isomerase, phosphoglycerate mutase, lactate dehydrogenase, glyceraldehyde phosphate dehydrogenase, aldolase, creatine kinase, enolase, phosphoglucose isomerase, pyruvate kinase, phosphoglucomutase, and phosphorylase b.

The relative concentration of the identified sarcoplasmic proteins was 83.6% of all sarcoplasmic proteins extracted from the pork meat. [19]

Conclusion

This article systematizes modern methodological approaches to electrophoretic separation and analysis of muscle proteins. It is shown that the evolution of methods — from free electrophoresis and one-dimensional SDS-PAGE to high-resolution two-dimensional technologies (2D-PAGE, 2D-DIGE) and capillary gel electrophoresis — has significantly expanded the possibilities of muscle biochemistry. Special attention was paid to modifications aimed at improving the separation of difficult-to-separate components: the use of gradient gels, the addition of methanol, pulse electrophoresis, and the optimization of buffer composition, which allowed for the clear separation of isoforms of heavy and light chains of myosin, tropomyosin, troponins, and other regulatory proteins.

References

1. Kielkopf CL, Bauer W, Urbatsch IL. Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis of Proteins. Cold Spring Harb Protoc. 2021;2021(12):10.
2. Hajba L, Guttman A. Ionic liquids in capillary electrophoresis analysis of proteins and carbohydrates. J Chromatogr A. 2024;1716:464642.
3. Sule R, Rivera G, Gomes AV. Western blotting (immunoblotting): history, theory, uses, protocol and problems. Biotechniques. 2023;75(3):99-114.
4. Bhimwal R, Rustandi RR, Payne A, Dawod M. Recent advances in capillary gel electrophoresis for the analysis of proteins. J Chromatogr A. 2022;1682:463453.
5. Fritz JD, Swartz DR, Greaser ML. Factors affecting polyacrylamide gel electrophoresis and electroblotting of high-molecular-weight myofibrillar proteins. Anal Biochem. 1989;180(2):205-210.
6. Reiser PJ, Belevych N, Shope L, Hanaoka B. Methanol gel electrophoresis: Separation of human fast and slow myosin light chain 1 and other myofibrillar protein isoforms on a single gel format. ELECTROPHORESIS. 2024 Oct;45(19-20):1851-1859.
7. O'Brien GA, Corbett JM, Dunn MJ, Cumming DV, May AJ, Yacoub MH. Electrophoretic analysis of electrically trained skeletal muscle. Electrophoresis. 1992;13(9-10):726-728.
8. Murphy S, Dowling P, Ohlendieck K. Comparative Skeletal Muscle Proteomics Using Two-Dimensional Gel Electrophoresis. Proteomes. 2016;4(3):27. Published 2016 Sep 9.
9. Di Luca A, Hamill R, Mullen AM, Elia G. DIGE Analysis of Animal Tissues. Methods Mol Biol. 2023;2596:201-216.
10. Ахремко Анастасия Геннадьевна. "ПРИМЕНЕНИЕ МЕТОДА ДВУМЕРНОГО ЭЛЕКТРОФОРЕЗА ДЛЯ ОЦЕНКИ СОСТАВА МЯСНЫХ ПРОДУКТОВ НА ПРИМЕРЕ МОДЕЛЬНЫХ ФАРШЕЙ" Все о мясе, no. 3, 2019, pp. 46-48.
11. Tomioka Y, Arakawa T, Akuta T, Nakagawa M, Ishibashi M. Analysis of proteins by agarose native gel electrophoresis in the presence of solvent additives. Int J Biol Macromol. 2022;198:26-36.
12. Bárány K, Bárány M, Giometti CS. Polyacrylamide gel electrophoretic methods in the separation of structural muscle proteins. J Chromatogr A. 1995;698(1-2):301-332.
13. Bamman MM, Clarke MS, Talmadge RJ, Feeback DL. Enhanced protein electrophoresis technique for separating human skeletal muscle myosin heavy chain isoforms. Electrophoresis. 1999;20(3):466-468.
14. Porzio MA, Pearson AM. Improved resolution of myofibrillar proteins with sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Biochim Biophys Acta. 1977;490(1):27-34.
15. Kubis HP, Gros G. A rapid electrophoretic method for separating rabbit skeletal muscle myosin heavy chains at high resolution. Electrophoresis. 1997;18(1):64-66.
16. Sant'Ana Pereira JA, Greaser M, Moss RL. Pulse electrophoresis of muscle myosin heavy chains in sodium dodecyl sulfate-polyacrylamide gels. Anal Biochem. 2001;291(2):229-236.
17. Gelfi C, De Palma S. 2D DIGE analysis of protein extracts from muscle tissue. Methods Mol Biol. 2012;854:155-168.
18. Ohlendieck K. Comparative 3-Sample DIGE Analysis of Skeletal Muscles. Methods Mol Biol. 2018;1664:93-108.
19. Grujić Radoslav, and Savanović Danica. "Analysis of myofibrillar and sarcoplasmic proteins in pork meat by capillary gel electrophoresis" Foods and Raw materials, vol. 6, no. 2, 2018, pp. 421-428.

DOI:10.31579/2690-1897/313