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Review

Deciphering metabolic networks by blue native polyacrylamide gel electrophoresis: A functional proteomic exploration

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ABSTRACT

Metabolism is the consortium of reactions within a cell which directs a variety of processes including energy synthesis, signalling and the behaviour of a biological system. Metabolic networks, and more specifically the activity of enzymes within them, provide an accurate status of how cellular information is being executed. The performance of these networks and their ability to siphon metabolites in a number of directions may be the difference between a healthy and diseased state. Blue native polyacrylamide gel electrophoresis (BN-PAGE), owing to its simplicity and wide-ranging applications, permits the inspection of these nodules. The separation of proteins and enzyme complexes in their native format enables the exploration of enzymatic activity in metabolic networks via in-gel assays. These are quick, specific, and amenable to further studies. This electrophoretic technology not only enables the visualization of enzymatic efficacy but reveals the crosstalk among enzymes and their interactions with other organellar partners.

Contents

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1. Introduction

Metabolism is the foundation of all living organisms as it fulfils a multitude of crucial functions that dictate their survival, proliferation and in essence, existence. It is broadly divided into catabolism and anabolism. The latter involves the genesis of biomolecules and the former implicates the degradation of substrates [1]. These constitute the inner workings of an organism and are characterized by an array of metabolic networks that guides most cellular processes. These networks are orchestrated by enzymes that conjugate their efforts to bring a series of biochemical reactions to fruition [2,3]. For instance, glycolysis is dedicated to the catabolism of D-glucose with the effect of generating essential metabolites like ATP and pyruvate [4]. Depending on the needs of the cell, the latter may be shuttled to the tricarboxylic acid (TCA) cycle, a metabolic module which extracts reducing factors such as NADH and $FADH_2$ [5]. These enzymes often work in close association and are usually in close proximity to allow for the effective channelling of substrates and for proper regulatory control [6]. Therefore, probing such multi-enzymatic complexes requires non-invasive tools that will ensure the preservation of their activity [7].

Polyacrylamide gel electrophoresis is a technique that lends itself to the study of biomolecules in a wide range of fields, from biotechnology to molecular biology and forensic science [8]. Its ability to separate moieties such as proteins, lipids, and nucleic acids in native forms grants investigators the capacity to inspect these molecular components [9]. Indeed, great leaps in molecular medicine would not have been possible without this critical tool. By excluding strong detergents (e.g., sodium dodecyl sulfate) and utilizing Coomassie blue G-250 to induce a charge shift in proteins, blue native polyacrylamide gel electrophoresis (BN-PAGE) was originally developed as a means of separating membrane complexes in enzymatically active form [10]. However, modifications to this electrophoretic technique and the emergence of novel in-gel activity assays have allowed researchers to analyze a wider spectrum of enzymes, membrane proteins and many supra-complexes residing in biological compartments [11–13]. The modulation of acrylamide concentration and introduction of micelle-forming chemicals have extended the analytical flexibility of this technique [13,14]. The fine-tuning of this electrophoretic technique has permitted the investigation of key enzymatic activities in metabolic networks and has yielded precise information on complex cellular processes [8,15].

Although these entities may be probed individually, BN-PAGE provides a relatively non-invasive technique to explore how these biomolecules function collaboratively. The ability of this analytical tool to maintain proteins and their superstructures in native conditions enables the molecular visualization of these enzymes in a relatively undisturbed fashion [16]. This procedure also tends to have minimal impact on the transient forces that orchestrate diverse enzymes to congregate for their select metabolic tasks. Hence, the exploration of these complexes and some of the key enzymes that dictate the occurrence of a specific metabolic network generate an accurate functional landscape of a particular metabolic nodule and its performance at a given time [17].

2. Energy-generating networks

As the primary generator of the universal energy currency adenosine triphosphate (ATP) in aerobic organisms, the status of oxidative phosphorylation and the electron transport chain (ETC) complexes provide a strong gauge of energetic metabolism and mitochondrial well-being [18]. NADH and $FADH_2$ provide the reducing power necessary to generate a proton gradient in complexes I–IV across the internal mitochondrial membrane. In turn, complex V harnesses this gradient to catalyze the formation of the high-energy compound ATP from adenosine diphosphate (ADP) [19]. The need to study the activity of ETC components arose from the identification of a number of neuromuscular disorders stemming from faulty mitochondrial activity. Genetic mutations in the oxidative phosphorylation complexes affect 1 in 5000 individuals, and would be troublesome to characterize without specialized enzyme assays [20,21]. In addition, environmental factors, such as metal stress, reactive oxygen and nitrogen species (ROS and RNS, respectively) are capable of disrupting this essential ATP-making machinery [22,23]. The inherent difficulty in studying ETC proteins lies in their hydrophobicity and highly complex structures. A strong detergent such as SDS leads to the dissociation of these multi-subunit structures, rendering activity assays ineffective [24].

To counter this, Schägger and von Jagow employed the mild detergent *n*-dodecyl β-D-maltoside (DDM), thus rendering the complexes soluble for electrophoresis and maintaining their superstructure [10]. Variations to the standard protocol, such as the substitution of digitonin for DDM, as well as the development of clear native PAGE, has led to the identification of all the ETC complexes [13]. Once separated, the activity of this ATP-generating machinery can be elucidated via specific in-gel enzymatic activity assays [11]. The formation of reducing cofactors like NADH, NADPH and $FADH_2$ can be readily visualized by the generation of formazan at the site of enzymatic activity in the presence of phenazine methosulfate (PMS) and iodonitrotetrazolium (INT) salts [25]. The substitution of PMS with 2,6-dichlorophenolindophenol (DCPIP) permits the monitoring of the enzymatic oxidation of reduced cofactors [26,27]. In some cases, products which are not amenable to redox processes can be coupled with redox enzymes in order to reveal their localization in the gel (Fig. 1) [26–28].

For instance, complexes I (EC 1.6.5.3) and II (EC 1.3.5.1) can be detected via the addition of their preferred substrates (NADH and succinic acid, respectively) and an indicator such as PMS coupled to INT [11,14]. The latter, when reduced, produces a pink-coloured formazan precipitate at the location of the complex in-gel. A reaction mixture consisting of cytochrome C and the indicator 3.3′-diaminobenzidine is used to pinpoint complex IV (EC 1.9.3.1), while ADP and $Pb(NO_3)_2$ are used to observe complex V (EC 3.6.3.14) [11,14,29]. These activity assays permit the qualitative comparison of ETC activity between disparate conditions, such as a healthy and diseased state. While semi-quantitative measurements are feasible using densitometric software such as ImageJ, precise