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Blue native polyacrylamide gel electrophoresis and the monitoring of malate- and oxaloacetate-producing enzymes

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Abstract

We demonstrate a facile blue native polyacrylamide gel electrophoresis (BN-PAGE) technique to detect two malate-generating enzymes, namely fumarase (FUM), malate synthase (MS) and four oxaloacetate-forming enzymes, namely pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PEPCK), citrate lyase (CL) and aspartate aminotransferase (AST). Malate dehydrogenase (MDH) was utilized as a coupling enzyme to detect either malate or oxaloacetate in the presence of their respective substrates and cofactors. The latter four oxaloacetate-forming enzymes were identified by 2,6-dichloroindophenol (DCIP) and *p*-iodonitrotetrazolium (INT) while the former two malate-producing enzymes were visualized by INT and phenazine methosulfate (PMS) in the reaction mixtures, respectively. The band formed at the site of enzymatic activity was easily quantified, while Coomassie staining provided information on the protein concentration. Hence, the expression and the activity of these enzymes can be readily evaluated. A two-dimensional (2D) BN-PAGE or SDS-PAGE enabled the rapid purification of the enzyme of interest. This technique also

Abbreviations: BN-PAGE; blue native polyacrylamide gel electrophoresis; FUM; fumarase; MS; malate synthase; AST; aspartate aminotransferase; PC; pyruvate carboxylase; PEPCK; phosphoenolpyruvate carboxykinase; CL; citrate lyase; MDH; malate dehydrogenase; 2D BN-PAGE; two-dimensional blue native polyacrylamide gel electrophoresis; SDS-PAGE; sodium dodecyl sulfate; 1D; one-dimensional.

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provides a quick and inexpensive means of quantifying these enzymatic activities in normal and stressed biological systems.

Keywords: BN-PAGE; Oxalate; Malate; Enzymes; Activity

1. Introduction

Enzymes involved in the synthesis of malate and oxaloacetate form a pivotal aspect of cellular metabolism in all organisms [1]. They help generate essential precursors that are involved in the production of energy, in gluconeogensis, in lipogenesis, and in the metabolism of amino acids. PC [E.C. 6.4.1.1], that mediates the formation of oxaloacetate from ATP, HCO_3^- and pyruvate, is a key enzyme that funnels metabolites towards the production of glucose [2]. CL is a lipogenic enzyme as it enables the transport of acetyl-CoA into the cytoplasm for its interaction with acetyl-CoA carboxylase. The formation of malonyl-CoA from acetyl-CoA, ATP and HCO_3^- is a critical step in the formation of fatty acid in most organisms [3]. The oxaloacetate generated by this cleavage is also essential in the production of NADPH, an essential ingredient for lipogenesis. AST [E.C. 2.6.1.1] is an enzyme that generates oxaloacetate via the transamination of aspartate and plays an important role in cellular nitrogen homeostasis [4]. PEPCK [E.C. 4.1.1.49], critical in linking the TCA cycle to gluconeogenesis, mediates the formation of phosphoenolpyruvate from oxaloacetate. This enzyme is crucial in carbohydrate metabolism [5]. FUM [E.C. 4.2.1.2], on the other hand, is part of the tricarboxylic acid (TCA) cycle and is involved in the reversible hydration of fumarate to malate. The malate is subsequently oxidized to generate NADH that eventually propels the synthesis of ATP in the electron transport chain [6]. Together with isocitrate lyase, MS [E.C. 2.3.3.9] forms the glyoxylate shunt. This metabolic network contributes to the survival of organisms exposed to nutrient and/or metal stresses by preventing the loss of carbon via the TCA cycle and in aiding the synthesis of oxalic acid [7–9].

Hence, it is very important to devise simple and inexpensive techniques to study these enzymes that cover a broad range of metabolic functions. Although spectrophotometric methods have been routinely utilized to monitor activities of these enzymes, interfering reactions hinder the accuracy of these assays. Sensitivity is relatively low and high quantities of proteins are required [4,10]. To quantitate the expression of these enzymes, antibodies that are expensive and/or not readily available have to be used. The procedure described here allows the monitoring of both enzymatic activity and relative protein expression without necessitating the use of antibodies.

Although BN-PAGE has been widely reported in the study of mitochondrial membrane proteins [11], we have adapted this technique to monitor both soluble and membrane-bound enzymes involved in the production of either malate or oxaloacetate. MDH is utilized as the coupling enzyme while INT is the detecting chromophore. In this report, we describe how BN-PAGE can detect, quantitate and purify six enzymes involved in malate