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Notes & Tips

Dimethylformamide interferes with Coomassie dye staining of proteins on blue native gel electrophoresis

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ABSTRACT

Blue native gel electrophoresis (BN–PAGE) is used extensively for characterization of mitochondrial respiratory complexes and uses the binding of Coomassie brilliant blue G-250 to visualize proteins. Oxidative modification of sulfhydryl groups of such proteins can be evaluated by labeling with iodoacetamide conjugated to biotin (BIAM) and detected with streptavidin peroxidase on Western blots following BN–PAGE. However, dissolving BIAM in dimethylformamide, a recommended solvent, reduces Coomassie blue G staining to proteins during BN–PAGE. This interference is prevented by dissolving BIAM in dimethyl sulfoxide. Precautions in the use of the dye for protein staining subsequent to BIAM labeling are discussed.

Coomassie brilliant blue G-250 (CBB G-250),[1] a dye that binds to proteins through electrostatic and hydrophobic interactions and Van der Waal's forces [1], is routinely used in the estimation of proteins. CBB G-250 is a disulfonated triphenylmethane compound and exists in three ionic species: cationic, neutral, and anionic [2,3]. The binding of the neutral species of CBB G-250 to proteins is through both hydrophobic and electrostatic interactions, whereas anionic CBB G-250 binds to proteins mainly through electrostatic attractions. This is through the dissociated sulfonic groups of CBB G-250 and basic amino acids (arginine, lysine, and histidine) on proteins. Sulfonic groups of cationic CBB G-250 are protonated, preventing their ionic binding to proteins [4].

Blue native gel electrophoresis (BN–PAGE) is a method for the separation of intact protein complexes in their native form and has been used extensively to separate individual mitochondrial electron transport chain complexes. CBB G-250 is routinely used in BN–PAGE, where it binds to proteins and gives them a net negative charge that allows for their electrophoretic separation [5].

Free thiol groups of cysteine moieties on proteins are susceptible to posttranslational oxidative and nitrosative modification induced by reactive oxygen species (ROS) and reactive nitrogen species (RNS), respectively [6,7]. Iodoacetamide conjugated to biotin (BIAM) is a reagent that has been widely used to identify protein thiol oxidation in redox biology [8,9] due to the ability of iodoacetamide to bind to reduced thiols but not oxidized or modified thiols on proteins. These labeled proteins can then be visualized on immunoblots using streptavidin conjugated to horseradish peroxidase, where proteins containing modified thiol groups can be identified by their decreased labeling with the BIAM reagent.

During experiments to evaluate sulfhydryl group modifications of mitochondrial electron transport chain complexes, BN–PAGE of BIAM-labeled mitochondrial samples was carried out. Mitochondrial pellets from rat muscle (500 μg protein) were solubilized in 80 μl of extraction buffer (containing 0.75 M aminocaproic acid and 50 mM Bis–Tris), followed by the addition of 12.0 μl of 10% lauryl maltoside. Samples were then incubated on ice for 20 min and centrifuged at 14,000 rpm (18,000*g*) for 5 min [5,10,11]. An aliquot of the supernatant containing extracted mitochondrial complexes (150 μg protein) was incubated with 100 μM BIAM dissolved in dimethylformamide (DMF) (final concentration) for 15 min at 25 °C in the dark. The final concentration of DMF in the mixture was 1.29 M. The labeling reaction was terminated with 20 mM β-mercaptoethanol (final concentration). Mitochondrial extracts labeled with BIAM and those incubated with only 20 mM β-mercaptoethanol were treated with 3 μl of 5% CBB G-250 in 0.5 M aminocaproic acid. Volumes corresponding to 150 μg of protein were then subjected to BN–PAGE (5–12% gradient gels). Gels were imaged after the run without further staining.

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[1] *Abbreviations used:* CBB G-250, Coomassie brilliant blue G-250; BN–PAGE, blue native gel electrophoresis; ROS, reactive oxygen species; RNS, reactive nitrogen species; BIAM, iodoacetamide conjugated to biotin; DMF, dimethylformamide; DMSO, dimethyl sulfoxide.

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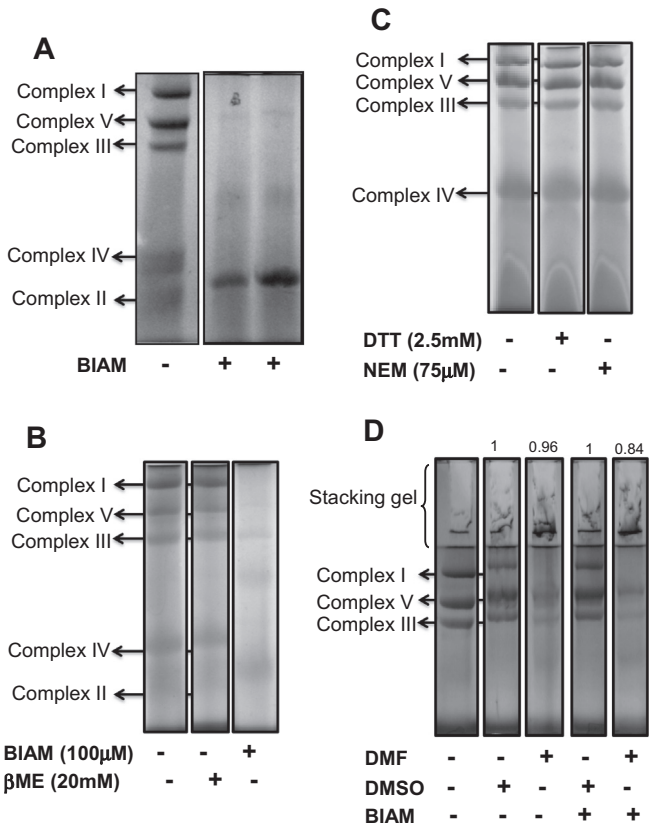

Fig.1. BN–PAGE separation of mitochondrial electron transport chain complexes under various conditions. (A) Samples separated with or without labeling with BIAM (in DMF). (B) Samples subjected to BN–PAGE after labeling with BIAM (in DMF) or 20 mM β-mercaptoethanol (βME) alone. (C) Samples separated after treatment with either 2.5 mM dithiothreitol (DTT) or 75 μM *N*-ethylmaleimide (NEM). (D) Mitochondrial samples subjected to BN–PAGE with or without labeling with either BIAM (in DMF) or BIAM (in DMSO). Numbers above lanes indicate total protein intensity as fold change when compared with DMSO alone.

A significant reduction of CBB G-250 staining of proteins in the BIAM-treated samples compared with untreated samples was observed (Fig. 1A). This effect is not due to the β-mercaptoethanol added to stop the BIAM labeling reaction given that samples subjected to BN–PAGE after treatment with 20 mM β-mercaptoethanol show bands of CBB G-250 staining intensity similar to untreated samples (Fig. 1B). To rule out an effect of sulfhydryl group modification per se on CBB G-250 protein staining, samples were treated with either *N*-ethylmaleimide (75 μM [final concentration] dissolved in water and incubated with samples on ice for 30 min), which blocks free sulfhydryl groups [12], or dithiothreitol (2.5 mM [final concentration] dissolved in water and incubated with sample at 37 °C for 30 min), which reduces sulfenic acid or disulfides to thiol groups [13], and then were subjected to BN–PAGE. As can be seen in Fig. 1C, neither *N*-ethylmaleimide nor dithiothreitol interfered with CBB G-250 binding to protein.

BIAM can be dissolved in either DMF or dimethyl sulfoxide (DMSO) as per the manufacturer's instructions (Ana Spec, Fremont, CA, USA; Biotium, Hayward, CA, USA; or Invitrogen, Carlsbad, CA, USA). To assess whether these solvents affect CBB G-250 binding to protein, samples were treated with either DMF or DMSO alone for 15 min at 25 °C in the dark and then were subjected to BN–PAGE. Treatment with DMF alone inhibited dye binding to proteins, whereas BIAM dissolved in DMSO did not, showing bands of dye intensity similar to untreated samples (Fig. 1D). Quantitation of total protein intensity in each lane showed similar levels, confirming equal protein loading.

A possible reason for the better protein staining in the case of DMSO could be that the higher dielectric constant and solvating nature of DMSO prevents CBB G-250 precipitation, resulting in better dye binding to the protein when compared with DMF [14]. The lower CBB G-250–protein binding of samples treated with BIAM–DMF would also result in a lower negative charge on proteins, leading to their retention in the sample wells (as seen in Fig. 1D), and interfere with their separation during BN–PAGE. Thus, these studies indicate that in experiments to characterize sulfhydryl groups on proteins, BIAM should be dissolved in DMSO, and not DMF, to prevent interference with CBB G-250–protein binding, which is critical for BN–PAGE separation.

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