

Counting the Integral Number of Sulfhydryl Groups in Bakers' Yeast Aldehyde Dehydrogenase Using Urea-Polyacrylamide Gel Electrophoresis

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Abstract: Aldehyde dehydrogenase is a polymorphic enzyme responsible for the oxidation of aldehydes to carboxylic acids, which leave the liver and are metabolized by the body's muscle and heart. This study aimed at investigating the number of sulfhydryl groups present in aldehyde dehydrogenase. Urea Polyacrylamide Gel Electrophoresis (Urea-PAGE) approach was used to count the integral number of sulfhydryl groups present in Bakers' yeast aldehyde dehydrogenase. Aldehyde dehydrogenase solution was sequentially denatured, reduced and alkylated using urea, dithiothreitol (DTT), iodoacetamide and iodoacetic acid solutions. This was subsequently analysed on 8.0 M Urea-PAGE at 100 v for 4 hours and stained by fast Coomassie method. A single band on the 8.0 M Urea-PAGE indicates that the enzyme, aldehyde dehydrogenase, contains one disulfide bond. This disulfide bond present in the enzyme might contribute to the stability of the enzyme.

Keywords: Aldehyde dehydrogenase, Urea-PAGE, Bakers' yeast, disulfide, stability.

I. INTRODUCTION

Aldehyde dehydrogenase (ALDH) is a group of dehydrogenase enzymes that catalyze the oxidation or dehydrogenation of aldehydes. ALDH is a polymorphic enzyme [1] responsible for the oxidation of aldehydes to carboxylic acids, which leave the liver and are metabolized by the body's muscle and heart.

ALDH from bakers' yeast (*Saccharomyces cerevisiae*) is a tetramer consisting subunits of approximately 57 k Da, 114 k Da, 171 k Da and 228 k Da respectively. The monomers are thought to be assembled into tetramer in a heterologous square arrangement [2]. The enzyme can catalyze the oxidation of a wide range of substrates, including: acetaldehyde, formaldehyde, propionaldehyde, n-butylaldehyde, isobutylaldehyde, n-valeraldehyde, caproaldehyde, benzaldehyde, glycoaldehyde, malonicsemialdehyde, D-glyceraldehyde, and succinic aldehyde [3].

A method was developed by Creighton (1980) to count integral numbers of amino acid residues which is particularly useful for the determination of cysteine residues. ALDH characterized to date are sensitive to various sulfhydryl modifying reagents. In the case of cysteine, the method relies on charge differences introduced by specific chemical modification of the amino acid, using either a single reagent or the competition between two or more different reagents [4], [5]. The reactivity of free sulfhydryl groups make them useful in stability, metal binding, nucleophilicity and catalytic activity. Two cysteine residues form a disulfide bond which is useful in protein folding, stabilization and regulation of its activity [6].

II. MATERIALS AND METHODS

Materials

Ethylenediaminetetraacetic acid (EDTA), Trisma-base, Tris-HCl, dithiothreitol (DTT), Urea, Iodoacetic acid, Potassium hydroxide (KOH), Acrylamide, Bis-acrylamide, N,N,N',N'-tetramethylethylenediamine (TEMED), ammonium persulfate, agrose, bromophenol blue, Acetic acid, Methanol, glycerin, aldehyde dehydrogenase (Bakers' yeast) are all products of Sigma Chemical Company, USA. All chemicals used were of analytical grade. Distilled water was used throughout the experiment.

Methods

i. Denaturing and reduction of Aldehyde dehydrogenase

10 μ L 1.0 M Tris-HCl pH 8.0, 0.1 M EDTA, 1.0 M DTT and 1.0 mL of 8.0 M Urea solution were added to 0.2 mg of the enzyme. The solution was mixed and incubated at 37^oC for 30 minutes.

ii. Alkylation of Aldehyde dehydrogenase

During this period of denaturation and reduction, the stock solution of the alkylating reagents were prepared. Reagent A was prepared by dissolving 46.5 mg of Iodoacetamide (IAM) in 1.0 mL of 0.25 M Tris-HCl (pH 8.0). Reagent B was prepared by dissolving 46.5 mg of Iodoacetic acid in 0.25 mL of 1 M KOH and 0.75 mL of 0.33 M Tris-HCl (pH 8.0). 50 μ L each of reagents A and B were mixed together to form reagent C. Reagent D contained a mixture of 150 μ L of A and 50 μ L of B while reagent E was a mixture of 450 μ L of A and 50 μ L of B. 10 μ L of solutions A,B,C,D,E was added into five tubes (tube 1- tube 5) respectively. 40 μ L of the unfolded reduced protein solution was added to each of the tubes 1-5. The content of the tubes were gently mixed twice with Eppendorf pipette and left at room temperature for 15 minutes and stored on ice. Another tube 6 was labelled and an aliquot of 10 μ L from each of the tubes 1-5 was pipetted into it and mixed gently. The solutions in tubes 1-6 were analyzed on Urea polyacrylamide gel. This method relies on the charge differences introduced by specific chemical modification of the amino acid, using either a single reagent or two or more reagents. As the ionic charge is altered, the separation of the species with different numbers of the modified groups can be readily achieved by electrophoresis.

iii. Preparation of 20 mL of 8.0 M urea 11% acrylamide gel

9.8g of Urea was weighed and 3.15 mL of distilled water was added. 3.65 mL acrylamide solution was later added. 1 mL of 1.5 M Tris-HCl pH 8.0 was added and then 2.5 mL Ammonium persulfate solution. The mixture was allowed to completely dissolved and degassed. 1 mL of TEMED was pipette into the mixture. The entire solution was thoroughly mixed and immediately poured into the gel apparatus 10 cm x 10.5 cm. The thickness of the spacer used was 1.5 mm. This was left overnight to polymerize.

iv. Urea Polyacrylamide gel electrophoresis

The samples (tubes 1-6) were prepared for electrophoresis by the addition of Coomassie brilliant blue. 20 μ L of each sample was layered into the wells of the gel. Electrophoresis was run at a constant voltage of 100 v for 4 hours at room temperature.

v. Staining and De-staining

At the end of the electrophoresis, the gel was removed and immersed into a protein stain (0.25g Coomassie brilliant blue mixed with 125 mL methanol, 25 mL Acetic acid and 100 mL distilled water) for several hours with gentle agitation. The gel was washed in several changes of de-staining solutions (250 mL methanol and 50 mL Acetic acid made up to 500 mL with distilled water).

III. RESULTS

The denaturation, reduction and alkylation of Aldehyde dehydrogenase (ALDH) using Urea, DTT and Iodoacetamide and Iodoacetic acid followed by 8.0 M Urea Polyacrylamide gel electrophoresis yielded one protein band. A single band indicates one disulfide bridge per molecule in 8.0 M Urea PAGE.

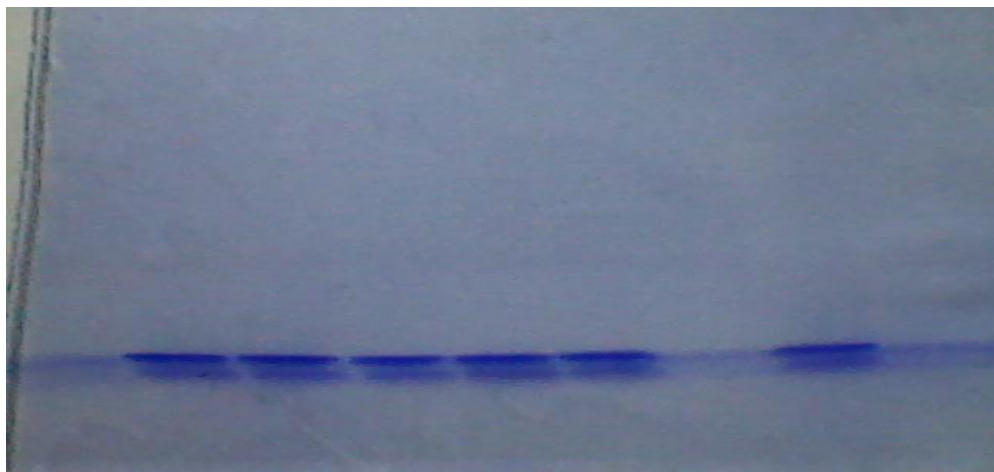


Figure 1: Determination of the number of disulfide bond in Aldehyde dehydrogenase.

The protein sample was denatured and reduced with 10 μ L of each of 1.0 M Tris-HCl pH 8.0, 0.1 M EDTA, 1.0 M DTT and 1.0 mL of 8.0 M Urea solution. This was incubated at 37^oC for 30 minutes. 40 μ L of the unfolded protein solution was added to 5 tubes each. Tube A contained 46.5 mg Iodoacetamide (IAM), tube B Iodoacetic, mixture of A and B formed C, 150 μ L of A with 50 μ L of B formed D while 450 μ L of A with 50 μ L of B formed E. These mixtures were left at room temperature for 15 minutes. 10 μ L of each sample was applied to 8.0 M Urea PAGE (lanes 1-5). Equal amounts of samples were mixed and subjected to 8.0 M Urea PAGE (lane mixture). Electrophoresis was carried out for 4 hours, 100 volts at room temperature. Gel was stained for proteins with Coomassie brilliant blue. The single band shows one disulfide bond. The figure shows electrophoretic mobility of denatured and reduced Aldehyde dehydrogenase treated with IAA and/or IAM.

IV. CONCLUSION

To study the number of disulfide bridges in Aldehyde dehydrogenase molecule a simple procedure developed by Creighton and Hollecker was applied [4], [5]. It is based on chemical modification of cysteine residues of the analyzed protein sample with IAA or IAM or the mixture of both. These reagents added negatively charged molecule to the protein. The result shows that Aldehyde dehydrogenase from Bakers' yeast is an enzyme containing one disulfide bond. That is, only 2 of its cysteine residues were responsible for the formation of the disulfide bond, others were exposed. This is similar to the number of disulfide bonds found in L-threonine dehydrogenase from *E. coli* which had a total of 6 half-cysteine residues in which only 2 existed in disulfide linkage. Pea NADP Malate dehydrogenase also gave similar result as reported by Scheibe *et al* [7]. The native oxidized enzyme was characterized by an extremely slow reactivity of two thiols. The two sulfhydryl residues forming the disulfide bond in Aldehyde dehydrogenase might serve as a center for ligand binding that enhance the activity of the enzyme. Hence, only two cysteine residues (among other cysteine residues not covered by this study) formed the disulfide linkage in the enzyme aldehyde dehydrogenase.

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