Thermal Denaturation of Proteins for SDS-PAGE Analysis by Microwave Irradiation

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One of the most useful tools available to researchers is sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (3). It is a rapid and relatively simple method for resolving proteins and is routinely used to monitor proteins during purification, assess expression levels, estimate molecular weights and characterize multimeric proteins. A number of variations exist, but the method developed by Laemmli (5) is the most widely used.

Sample preparation for SDS-PAGE analysis consists of denaturing the sample with heat in the presence of SDS and a reducing agent such as 2-mercaptoethanol (2-ME) or dithiothreitol (DTT). This is achieved by mixing the sample with an appropriate volume of concentrated sample buffer (10). The heating step, usually 3–5 min at 100°C, can be performed by incubating the sample in a microcentrifuge tube in either boiling water or a heating block set at 100°C. This treatment destroys secondary and tertiary structure, solubilizes the protein, dissociates polypeptides and reduces disulfide bonds.

There are safety drawbacks in both of these heating methods. When microcentrifuge tubes are heated to 100°C, the entire contents become heated, often resulting in the caps popping open as a result of pressure building up from the heated air within the tube. This can be particularly hazardous if the contents of the tube are radiolabeled, causing aerosols or even droplets of radioactive material to be spread over the surrounding area. Methods for overcoming the build-up of pressure include puncturing the cap of the tube with a needle, introducing another potential hazard, or the use of more expensive screw-cap tubes. In addition, conventional methods of heating result in the whole tube becoming hot, making handling difficult.

We describe here a microwave-based heat denaturation method. The use of microwaves in laboratories has become commonplace, and they are regularly used to boil agarose, to melt agar and to warm serum and media before use in cell culture. Other applications include the rapid thawing of blood components (8), protein assays (1), fixing of samples for electron microscopy and immunohistochemistry (6), in situ end-labeling of DNA (7) and preparing plasmid (4,11) and genomic (2,9) DNA.

To demonstrate the efficiency of microwave irradiation in the preparation of protein samples for SDS-PAGE analysis, we have used purified bovine γ-globulins (Sigma Chemical, Poole, England, UK) for the comparison of the microwave and heating-block methods of protein denaturation. Aliquots (25 µL) of a 0.25 mg/mL solution of γ-globulins were placed into 1.5-mL microcentrifuge tubes and mixed with an equal volume of 2× SDS-PAGE sample buffer [0.0625 M Tris-HCl, pH 6.8, 15% (wt/vol) glycerol, 3.5% (wt/vol) SDS, 5% (wt/vol) 2-mercaptoethanol and 1.25% (wt/vol) bromophenol blue]. Controls were incubated for 3 min at either 37°C in a water bath or at 100°C in a heating block. As the negative control, 37°C was used (i.e., not boiled) in order to illustrate that the denaturation was as a result of boiling and not the effect of the sample buffer. Other samples were heated for various times from 10–90 s in 10-s increments on high power in a domestic 800-W microwave oven with a turntable (Panasonic Model NN-5452BBPQ). Microcentrifuge tubes were supported in a plastic "hedgehog" style test-tube rack (Nalge, Rochester, NY, USA) placed in the center of the oven. Molecular-weight-size markers and 20 µL of each sample were loaded onto a 10% SDS-PAGE mini-gel and electrophoresed at 150 V until the dye front reached the separating gel and then at 200 V until the dye front reached the bottom of the gel. The gel was stained with Coomassie blue and destained with acetic acid/methanol.

Denaturation of γ-globulins by microwave irradiation is evident after 30 s and complete by 80 s (Figure 1). This is less than half the time typically used in other heat-denaturation procedures. Furthermore, only the liquid within the tube became hot, and sufficient pressure to pop open the lids was not produced. The tubes remained cool enough to be removed from the microwave oven by hand and very little evaporation was observed on the walls of the tubes.

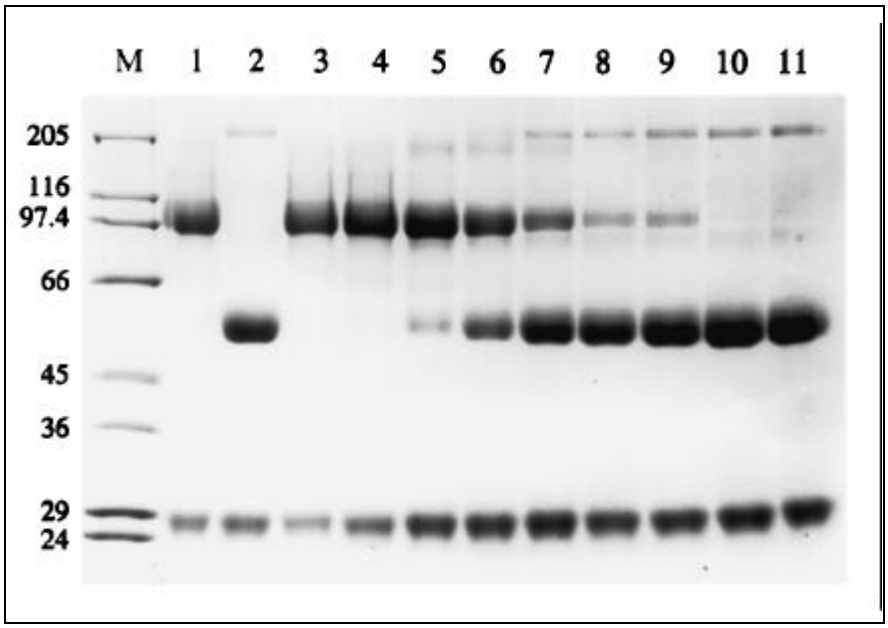

Figure 1. Thermal denaturation of γ-globulins by microwave irradiation. Protein samples (ca. 2.5 µg) were mixed with an equal volume of 2× SDS-PAGE sample buffer and heat denatured. Molecular weight markers (Sigma Chemical) were run in lane M, and the sizes of the markers (kDa) are indicated to the left of bands. Lane 1, 37°C for 3 min; lane 2, 100°C for 3 min; and lanes 3–11, increasing microwave irradiation for 10, 20, 30, 40, 50, 60, 70, 80 and 90 s, respectively. Heat denaturation of protein samples was achieved by 80 s (lane 10, cf. lane 2).

This experiment has been repeated with bluetongue virus (BTV) NS2 protein, BTV VP7 trimers, bovine serum albumin and human saliva, with results comparable to heating at 100°C for 3 min achieved in as little as 35 s (data not shown). It was also observed that the level of salt in microwaved solutions affected the time required to heat the solution, with higher salt concentrations lowering the heating time. The presence of moisture in the oven, usually as the result of evaporation during previous use, slightly increased the heating time.

We have shown that microwave irradiation of samples before SDS-PAGE is a viable and safe alternative to currently used methods for denaturation. An added benefit is the reduced time required to perform the heat-denaturation step of sample preparation.

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Alternative to Large Acrylamide Gels for DNA Analysis

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Large polyacrylamide gels such as those used for DNA footprinting, DNA protection and detection of cleavage chemistry have historically been used to separate small DNA fragments with base pair resolution (3). Typically, several titrations are required to establish optimal binding or cleavage conditions. In this report, a minigel is cast using sequencing grade acrylamide and sodium dodecyl sulfate (SDS). The SDS in the gel obviates the need for removal of proteins from the reaction, as often the presence of SDS in the stop buffer alone does not effectively dissociate the

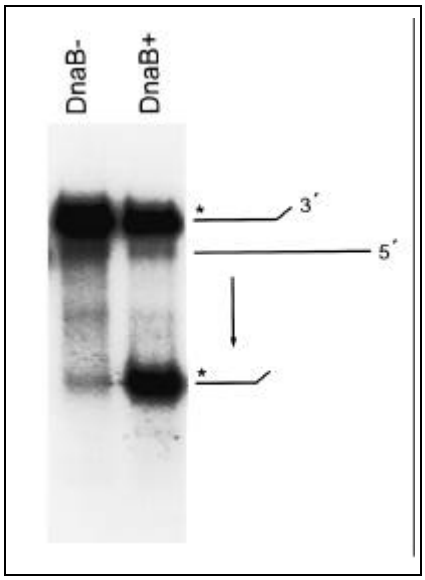

Figure 1. Helicase activity of DnaB using a synthetic partial duplex substrate. The gel depicts DnaB-catalyzed unwinding of a partial duplex substrate with a 20-nucleotide 5′ overhang. The radiolabel is indicated by an asterisk (*). Reactions were carried out in 20 mM Tris-HCl (pH 7.6) (ICN Biochemicals, Costa Mesa, CA, USA), 4% sucrose (ICN Biochemicals), 40 µg/mL bovine serum albumin (Boehringer Mannheim), 8 mM dithiothreitol (Boehringer Mannheim), 8 mM magnesium acetate (Sigma Chemical, St. Louis, MO, USA), 2.5 mM ATP (Boehringer Mannheim) and 0.5 pmol of ^{32}P end-labeled partial duplex substrate in a final volume of 25 µL. Reactions were initiated by the addition of DnaB (24 pmol monomer) and DnaC (24 pmol monomer) and were incubated at 30°C for 20 min. Reactions were stopped on ice with 5 µL of gel loading buffer and loaded directly onto the gel.