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THE ULTRASENSITIVE SILVER "PROTEIN" STAIN ALSO DETECTS NANOGRAMS OF NUCLEIC ACIDS

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Summary: The ultrasensitive silver staining procedure developed for proteins also stains nanogram quantities of RNA and DNA in polyacrylamide gels. A gradient polyacrylamide gel system is described which separates proteins from 10^4 to 10^6 M_r, RNA from 5S to 23S and DNA from 0.4 to 21 Kb. The sensitivity of nucleic acid silver staining in this gel system considerably exceeds that of commonly used DNA and RNA dye-binding stains.

Introduction: The highly sensitive silver staining technique, first developed by Switzer et al. (1) and recently modified by Oakley et al. (2) and by Merril et al. (3), has gained wide usage because it detects nanogram quantities of protein resolved in electrophoretic gels. Its sensitivity is about 100 times more than the commonly used Coomassie blue dye binding stain and is comparable to that of autoradiography of radiolabeled proteins.

The silver staining method has so far been assumed to be specific for proteins. In this paper, we report our findings that this method is also an ultrasensitive stain for DNA and RNA resolved in high-porosity polyacrylamide gels.

Materials and methods: Rabbit skeletal myofibrils were prepared by a modified procedure of Etlinger et al. (4) as described previously (5). Partially purified E. coli ribosomal RNA, prepared by the method of (6) was a gift of Dr. B. A. Hardesty. A total digest of λ DNA by Hind III-Eco R1 (7) was a gift of Dr. R. J. Meyer.

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate was performed on a 1 mm thick 9 x 15 cm slab gel consisting of 2-12% linear gradient (acrylamide:bisacrylamide = 50:1). The buffers are identical to Fairbanks et al. (8) except that 0.1% sodium dodecyl sulfate was used in gel and running buffers. Protein and nucleic acid samples were incubated with a sodium dodecyl sulfate sample buffer (8) at 50°C for 10-20 minutes before

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application to the gel slot (1 cm in width). Electrophoresis was done at 100 V (40 ma) for 2 hours.

Gels were silver-stained according to Oakley et al. (2) with a 30 minute soak in 50% methanol, 10% acetic acid, immediately after electrophoresis, followed by an overnight soak in 5% methanol, 7% acetic acid. Gels were then treated with 10% glutaraldehyde to "fix" protein bands in the gel matrix, followed by rinsing (2 hours) in distilled water. Ammoniacal silver solution was placed in the gel pan for 15 minutes before citrate-formaldehyde addition. Gels were gently agitated during each step. Upon addition of citrate-formaldehyde, silver deposition occurred immediately and intensified over a 3 minute period. Banding patterns were photographed on a light box every 10 seconds for 60-90 seconds using an Olympus OM-1 35 mm camera equipped with a Vivitar 55 mm/f2.8 macro lens.

Results: The polyacrylamide gradient slab gel system described here was developed in order to resolve proteins over a wide range in molecular weight. As shown in Fig. 1a, all major myofibrillar proteins from 15,000 to $>10^6$ daltons, are well resolved. The banding pattern revealed by silver staining is similar to that of Coomassie blue staining (at a much higher loading). However, the relative staining intensities are different for some bands when the two stains are compared (data not shown). A variation in color, ranging from a gold shade in some bands to a dark brown in others, was observed and was most marked immediately after citrate-formaldehyde addition. After the first minute of staining, however, the color variation vanished as all bands became medium brown.

The gel system also resolved RNA and DNA samples processed identically to the protein sample. As shown in Fig. 1 b-j, E. coli ribosomal RNA (23S, 16S and 5S) and all except two of the thirteen equimolar restrictive fragments of the λ DNA total digests were resolved. The silver staining procedure yielded brown bands well demarcated from background staining for both nucleic acids at extremely low loadings. The sensitivity of RNA staining, estimated from intensities of a series of sample loadings (data not shown) is one hundred to one thousand times more than that of methylene blue (9), depending on chemical composition. Fig. 1 b illustrates the relatively lower staining sensitivity of the 23S ribosomal RNA. Sensitivity of DNA silver staining, estimated in the same fashion, is approximately ten times

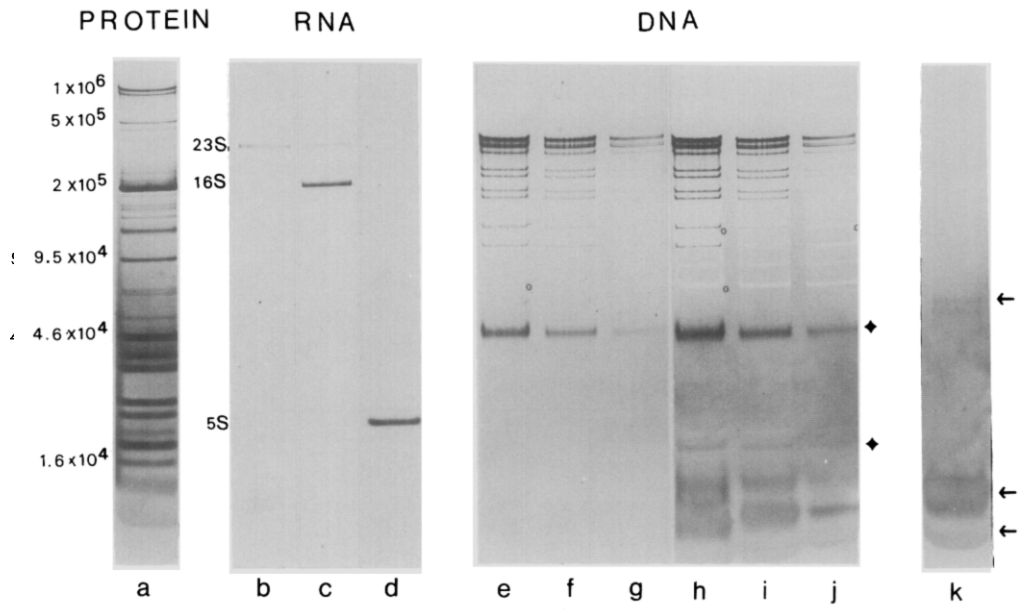

Fig. 1: Silver staining patterns of proteins, RNA and DNA resolved on sodium dodecyl sulfate-polyacrylamide gradient gels.

(a) Rabbit myofibrillar proteins (~2 μg); (b) 23S rRNA (~160 ng); (c) 16S rRNA (~125 ng); (d) 5S rRNA (~140 ng). (e)-(j) Total digest of λ DNA composed of the following Hind III-Eco RI restrictive fragments (in Kb): 21.6, 5.1, 4.95, 4.23, 3.45, 1.97, 1.88, 1.56, 1.35, 0.915, 0.825, 0.55 and 0.135. A total of twelve bands were resolved (all except the 5.1 Kb and 4.95 Kb pair). Three loadings of 240 ng (e,h), 130 ng (f,i), and 30 ng (g,j) of DNA were developed for 20 seconds (e-g) and for 60 seconds (h-j) in citrate-formaldehyde. Note the positive to negative transition (◦) and the protein bands (◆). (k): Sample buffer alone. Note the staining (←) of impurities.

more than that of ethidium bromide (10). Thus, nanogram quantities of nucleic acids can be easily detected. The color variation seen in protein staining was not apparent in the nucleic acid patterns; however, a DNA negative staining phenomenon occurred which was not observed in protein bands in the same gel. The negatively staining bands (see Fig. 1 h-j) originally appeared darker than background and bleached to transparency with time. This is illustrated in Fig. 1: lanes e-g were photographed 20 seconds after citrate-formaldehyde addition and lanes h-j 60 seconds after citrate-formaldehyde addition. The basis for negative staining is not clear but appeared to be more pronounced at low sample loading and longer developing time.

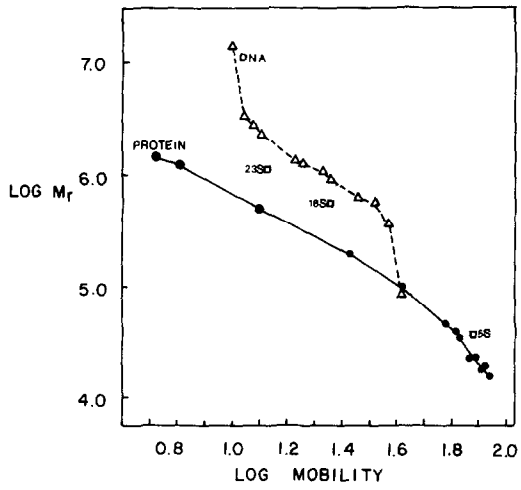

Fig. 2: Plots of log M_r versus log mobility for protein (●), RNA (□) and DNA (△). Mobility values were taken from Fig. 1.

The relative staining intensities of positively stained fragments do not appear to be proportional to their amounts in the equimolar mixture; presumably, this reflects the differing base compositions of fragments. The bands indicated by diamonds in Fig. 1 differed in color and definition from the upper DNA fragments and were presumed to be restriction enzymes because they were absent in undigested DNA and were not stained with ethidium bromide (R. Meyer, personal communication). The sensitivity and non-selective nature of silver stain is further demonstrated in Fig. 1 k where two diffuse staining bands near the bottom of the gel slot containing sample buffer alone are shown. The silver stain may have detected minor macromolecular impurities, detergent micelles or other anionic material in the sample buffer.

Fig. 2 compares mobilities of proteins, RNA and DNA in the gradient gel. The log M_r vs log mobility plot of proteins is roughly linear above 100,000 daltons, while that of DNA appears to be sigmoidal overall, and linear between 0.825 Kb and 5.1 Kb. Limited numbers of RNA sizes preclude the construction of a reliable curve.

Discussion: We have demonstrated that the silver staining in electrophoretic gels is not protein-specific. In fact, it is also an ultra-sensitive stain

for nucleic acids, detecting nanograms per band in polyacrylamide gels. The sensitivity of the staining technique is considerably higher than commonly used DNA and RNA detection methods.

A conclusion of practical importance is that caution is advised in interpretation of silver-stained "protein" samples which may actually contain RNA or DNA. This problem may be severe when crude cell extracts or subcellular fractions are analyzed on gels. A careful comparison of silver patterns with those of Coomassie blue, ethidium bromide and methylene blue, which preferentially (but not entirely specifically) stain protein, DNA and RNA respectively, may be necessary to draw definitive conclusions about the chemical nature of the bands. It should be noted that it is unlikely that nucleic acids would be expected to contribute to the silver staining pattern of two dimensional gels prepared according to O'Farrell (11), because the highly negatively charged nucleic acid fragments would be outside the common pH ranges in which proteins are resolved in isoelectric focusing gels.

The mechanism of silver staining of macromolecules is unknown. We suspect that the preferential binding of heavy metal ions such as silver and mercury to G-C base pairs may contribute in part to the staining (12). If so, the intriguing possibility exists that silver staining might be developed to reflect base composition of stained bands.

The simultaneous staining of proteins and nucleic acids in a single gel may find wide application in the structural studies of nucleoprotein assemblies.

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