Nonspecific Precipitation of Serum Proteins by Sodium Lauryl Sulfate in Agar Diffusion and Immunoelectrophoresis

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The anionic detergent sodium lauryl sulfate (SLS), in a final concentration of 0.1% and greater, reacted with whole serum in agar diffusion and immunoelectrophoresis to form artifactual precipitin lines. These lines occurred when either Ionagar or agarose was used as the supporting gel and were not affected by the presence of urea and 2-mercaptoethanol. Analytic chemical tests confirmed that the precipitating agent is SLS, and staining techniques showed that the detergent precipitates both protein and lipoprotein components of whole serum. Multiple artifactual precipitin lines occurred with a wide variety of animal sera, and a single line formed with human 7S immunoglobulin. Hence, in agar diffusion studies in which SLS is present in the test system, these artifactual lines may be easily misinterpreted as true antigen-antibody precipitin reactions.

In recent years, the anionic detergent sodium lauryl sulfate (SLS) has been used extensively in studies relating to protein composition and antigenic structure of viruses. A combination of SLS, 2-mercaptoethanol (ME), and urea (2) is commonly used to disrupt the tertiary folding of native viral proteins and to liberate polypeptide chains.

Electrophoresis of SLS-disrupted viral particles has revealed that different viruses contain characteristic sets of polypeptide chains. Further, SLS-solubilized proteins from a variety of plant and animal viruses have been shown to retain the antigenic property of reaction with antibody in a number of immunological systems, the most common being agar diffusion.

The present study shows that SLS reacts with serum proteins in agar diffusion and immunoelectrophoresis (IE). The artifactual precipitin lines and arcs formed by the reaction may easily obscure true viral antigen-antibody precipitin reactions, or worse, may actually be interpreted as specific reactions.

MATERIALS AND METHODS

SLS. SLS from three sources was used in this study: K & K Laboratories, Inc., Jamaica, N.Y.; Fisher Scientific Co., Fairlawn, N.J.; and Nutritional Biochemicals Co., Cleveland, Ohio. SLS from Nutritional Biochemicals Co. was analytically determined by that company to be 99.5% pure. The detergent was dissolved in 0.01 M sodium phosphate buffer (pH 7.2) at a concentration of 2.0%.

Sera. The sera tested were collected from rabbits and guinea pigs before and after they were immunized with several common respiratory viruses. Human 7S immunoglobulin was used to demonstrate reactions of SLS with a specific immunoglobulin. Additional sera tested were randomly selected normal and viral-immune sera from humans, mice, chickens, and horses; mouse ascitic fluid was also used.

Immunodiffusion. The double gel diffusion method was used (6). Supporting gel was either 0.5% Ionagar no. 2 (Colab Laboratories, Chicago, Ill.) in 0.85% NaCl or 0.7% agarose (Kallestad Laboratories, Minneapolis, Minn.) in 0.01 M phosphate buffer (pH 7.2) with 0.15 M NaCl and 0.01% Merthiolate. Wells were 3 mm in diameter and 9 mm apart, center-to-center, and were loaded one to three times. The plates were incubated for 1 to 3 days at 25 or 37 C in a moist chamber.

IE. IE was performed by the method of Scheidegger (9), with 1.0% Ionagar on microscope slides. The size of the antigen wells was modified to contain 0.02 to 0.03 ml of sample. Electrophoresis was carried out for 35 min at 250 v in Veronal buffer, pH 8.6. Arcs of precipitate developed after application of serum and incubation for 24 hr at 25 C.

Photography, staining, and sectioning of gels. Immunodiffusion plates and IE slides were photographed over indirect light; then they were washed and stained for protein and lipid (1) and photographed over direct light. Formed precipitin lines
photographed after SLS electrophoresis, tested for the chromatography from trometric sectioned, chromatograph-mass spectrometer in which the chromatograph column was maintained at 220 °C. Specific elution peaks were further examined by mass spectral analysis.

RESULTS

SLS consistently precipitated two to four serum proteins in reactions with whole guinea pig, rabbit, and human sera in double gel diffusion (Fig. 1). Precipitin lines became visible with most sera in approximately 2 hr; they were complete in 24 hr when incubated at either 25 or 37 °C with either normal sera or sera dialyzed for 24 hr against 500 volumes of 0.85% NaCl at 4 °C. Both diffuse and sharp lines were observed, and all stained positively for protein, lipid, or both. In general, the more concentrated the SLS antigen the more diffuse were the lines. Similar results were obtained when SLS alone or SLS-treated viral proteins were tested with human, rabbit, guinea pig, or mouse sera. Chicken and horse sera usually produced single lines and unconcentrated ascitic fluid produced none. Because of the presence of artifactual lines, we could not demonstrate that some SLS-disrupted viral antigens reacted specifically with homologous antisera in agar diffusion.

In contrast to results obtained with most of the whole sera used in these studies, a single precipitin line was formed when SLS was tested against purified human 7S immunoglobulin (Fig. 2). This line stained positively for protein and negatively for lipid; its position and shape were similar to that of precipitin lines formed in reactions of complete immunological identity. Visible lines occurred with SLS of a final concentration less than 0.2% against a constant...
concentration of 7.5 or 3.75 mg per ml of 7S immunoglobulin.

Two precipitin arcs were identifiable in the reaction of SLS or SLS-treated viruses with normal guinea pig serum in IE (Fig. 3). One arc stained positively for both lipid and protein and the other for protein only. The results of limited trials with a variety of SLS-treated viruses tested in IE against homologous antisera were identical with the results of tests involving these same antigens and nonimmune sera: in each case, the same two artifactual arcs formed. The inclusion of 1 M urea and 2% 2-mercaptoethanol in the SLS solution did not affect the formation of SLS-associated artifactual lines in either gel diffusion or IE.

Results of simple electrophoresis of 0.1% SLS indicated that the detergent migrated rapidly toward the anode. Manually sectioned gels showed that the sections with hemolytic activity for SRBC were coincidental with areas corresponding to precipitin arcs in immunoelectrophoresed companion gels. When formed precipitin lines from agar diffusion were manually sectioned from gels and eluted with distilled water, SLS was detected in the eluates by precipitation with BaCl₂ or KCl and by TLC. On Silica Gel G in three different solvent systems, TLC revealed that the major factor in the hydrolysate of material removed from the agar at the site of precipitin lines was identical to authentic lauryl alcohol. The hydrolysis product of authentic SLS was identical to both lauryl alcohol and the factor derived from the site of the precipitin line.

Similarly, gas chromatographic examination of the hydrolysate product and authentic lauryl alcohol resulted in identical elution patterns. The identity of the extracted hydrolytic product was further established by mass spectral data obtained from peaks eluted from the GLC column. The fragmentation patterns of the hydrolytic product and lauryl alcohol were, again, identical and the calculated molecular weights of both materials were consistent with lauryl alcohol. Therefore, we conclude that SLS is present at the site of the precipitin reaction in agar gels.

**DISCUSSION**

SLS is widely used in virology laboratories to disrupt viruses and release "masked" internal components for antigenic analysis and to solubilize viral proteins for separation by electrophoresis. SLS is effective for such procedures and its use as a solubilizing agent has contributed greatly to elucidation of the protein structure of plant and animal viruses. However, we find that results derived from studies that use SLS-treated antigens in agar diffusion must be interpreted with caution because the detergent reacts with serum proteins to produce artifactual precipitin lines in agar double-diffusion and IE. These lines are not removed by the usual washing procedures (1) and may easily be confused with antigen-antibody precipitin reactions.

SLS also reacts with human 7S immunoglobulin in agar double-diffusion to form a single sharp line of precipitation. This would indicate a priori that true antigen-antibody reactions may be obscured or blocked when SLS is present in the test system.

In IE the detergent migrates rapidly toward
the anode in the same manner as viral antigens. Addition of serum results in the formation of two precipitin arcs which stain for lipid or protein, or both. These staining properties indicate that SLS nonspecifically precipitates both protein and lipid components of whole serum. This finding is similar to that of Kunin and Tupasi (3), who found that dextran sulfate, a negatively charged, low-molecular-weight compound, formed a precipitate band with positively charged beta lipoproteins in whole serum.

Presence of SLS in formed precipitates or simple electrophoresis was initially detected by using hemolysis of SRBC as an indicator. In simple electrophoresis, hemolytic activity of eluates was coincidental to arcs in IE, and sectioned precipitates were lytic in the system. The finding that the detergent truly migrated in electrophoresis is further supported by the failure of eluates of the antigen wells after IE to lyse SRBC. SLS in formed precipitates was then confirmed by TLC and GLC experiments.

These results are compatible with other reports that SLS combines stoichiometrically with serum albumin and other proteins in precipitation reactions (5, 7, 8). Our data indicate that SLS-solubilized proteins and lipoproteins should be separated from the detergent if nonspecificity is to be avoided in agar diffusion studies. Prolonged dialysis against distilled water is effective in removing residual SLS from treated protein solutions, but the method is not entirely effective because of protein-SLS complex formation (5). SLS may also be precipitated from solution with KCl, low temperature (4), or BaCl₂ (7); alternately, proteins may be precipitated from solution with such common protein precipitants as ethanol or ammonium sulfate. However, none of these methods is entirely satisfactory because many viruses contain significant quantities of proteins and lipoproteins to which the detergent may also irreversibly bind. It is, therefore, evident that further investigation is needed into methods for adequately disassociating viral constituents from complexes with SLS before reliable agar diffusion studies of the antigenic structure of SLS-treated viral particles can be accomplished.

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