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Quantitative Immunoelectrophoretic Assay for Murine Oncornavirus p30: Noncovalent Facilitation by Sodium Dodecyl Sulfate

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Treatment of Rauscher murine leukemia virus lysates with the anionic detergent sodium dodecyl sulfate (SDS) at concentrations between 0.2 to 2.0% SDS per mg of viral protein greatly increased the anodal electrophoretic mobility of p30, the major internal polypeptide. SDS treatment did not reduce p30 antigenicity or cause nonspecific precipitation of normal serum proteins during subsequent immunoanalysis. The increased anodal electrophoretic mobility allowed assay of Rauscher murine leukemia virus p30 by Laurell rocket immunoelectrophoresis. An SDS-facilitated rocket immunoelectrophoresis assay is described that was highly reproducible (coefficient of variability, <3.0%) and capable of detecting 125 ng of viral protein. To our knowledge, this is the first report of a quantitative immunoelectrophoretic assay for an oncornavirus antigen. Since SDS binding is a general property of proteins, this method of noncovalently altering electrophoretic mobility appears to be applicable to other antigen-antibody systems.

Laurell "rocket" immunoelectrophoresis (RIEP; 13) is a sensitive quantitative immunoelectrophoretic (QIEP) technique in which immunoprecipitate rockets are formed by electrophoresing suitable antigens into gels containing specific antibody. The surface area and height of the precipitate is, generally, directly proportional to the amount of antigen present in the samples.

Although RIEP has received widespread use in quantitating a diverse array of antigens (2, 24), many antigens do not possess the electrophoretic mobility required to form characteristic rocket immunoprecipitates during RIEP analysis. Various methods of covalent chemical modification (6, 27) can be used to alter the pI's of proteins if a sufficient number of specifically modifiable amino acid residues are present. Other methods of increasing electrophoretic mobility must be used with antigens lacking sufficient amino acid residues for chemical modification.

Sodium dodecyl sulfate (SDS) is an anionic detergent that noncovalently confers a net negative charge to polypeptides, thereby minimizing their native charge differences (22, 23, 25). Because polypeptides bind identical amounts of SDS above an SDS-monomer concentration of 5×10^{-4} M (21, 25), we reasoned that SDS treatment might prove to be an effective method of noncovalently altering the electrophoretic mobilities of polypeptides that possess pI's generally unfavorable for RIEP quantitation.

The major internal polypeptide of Rauscher murine leukemia virus (RLV), p30 (1), possesses a pI of 6.7 (17) and does not readily permit quantitation by RIEP analysis due to its slow electrophoretic mobility. This report describes the successful use of SDS to confer a net negative charge on the polypeptide, thereby increasing its anodal electrophoretic mobility and allowing RIEP quantitative assay to be performed.

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MATERIALS AND METHODS

Virus. RLV was produced and purified from chronically infected JLS-V10 cell culture supernatants as previously described (12). Highly purified preparations (verified by SDS-polyacrylamide gel electrophoresis) were used in these investigations. Virus preparations generally contained 10^{12} virus particles and 2.0 mg of total protein per ml of concentrate. Protein was quantitated by the method of Lowry et al. (14) on samples previously disrupted with 0.1% Sarkosyl. Highly purified bovine serum albumin was used as the standard.

Antiserum. Antiserum against RLV p30 was ob-

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tained through the courtesy of J. Gruber, Office of Program Resources and Logistics, National Cancer Institute. The antiserum was prepared in a female goat by intramuscular injection of purified RLV p30 in Freund complete adjuvant. This antiserum yields a single immunoprecipitin band upon immunodiffusion and immunoelectrophoretic (IEP) analysis with lysed RLV and precipitates a single 30,000-dalton polypeptide from [^{3}H]leucine, [^{3}H]methionine, or [^{125}I]lactoperoxidase-iodinated RLV.

Immunological techniques. IEP was performed as directed by an IEP supply package obtained from Hyland Laboratories, Inc., Palo Alto, Calif. After electrophoresis of 5-μl samples, plates were developed with antiserum for 18 h at 37°C. RIEP and radial immunodiffusion (RID) were performed with the same Hyland kit by making necessary modifications. For RIEP, 25 ml of 2% agarose was poured into the kit's 7.5- by 13.5-cm lid to obtain a base of 2.4-mm thickness. Once hardened, a strip of agarose (7.5 by 8.0 cm) was removed from the center and replaced with a thinner, 1.25-mm layer (7.0 ml, total volume) of 2% agarose containing 5% anti-RLV p30 serum. Wells were punched with a 15-gauge needle immediately before electrophoresis. Two-microliter samples were subjected to electrophoresis at 10 mA per plate until bromophenol blue tracking dye migrated to the anodal well. RID was performed by pouring 15 ml of 2% agarose containing 5% anti-RLV p30 serum into a Hyland kit lid. Wells were punched with a 15-gauge needle, and 2-μl samples were placed in the wells for 24 h at 37°C. Immunoprecipitates were stained with 0.1% fast green before photography.

Lysis and SDS treatment. To insure that RLV p30 was available for reaction (i.e., that lysis of the virus was complete and p30 was solubilized), the RLV preparations used in this study were first lysed with 2% Nonidet P-40 and 2% sodium deoxycholate before SDS treatment. Though this treatment effectively solubilizes p30, it does not alter its electrophoretic mobility as compared to native p30 (unreported data).

SDS was obtained from Fisher Scientific Co., Pittsburgh, Pa., and further purified by extraction with acetone and chloroform. SDS was added to Nonidet P-40–deoxycholate RLV lysates as required from a 20% (wt/wt) stock solution to obtain desired concentrations. Treated samples were allowed to remain at room temperature for 30 min before immunoanalysis.

Because SDS binds to polypeptides, the amount of unbound SDS remaining in an SDS-protein mixture will be influenced by the amount of protein in the mixture. Therefore, the data in this report are presented as percentages of SDS per milligram of RLV protein, thereby reflecting that the total RLV protein in a solution must be considered when the percentage of SDS is reported.

Carbamylation. RLV preparations were carbamylated in the presence of 2% Nonidet P-40–deoxycholate by the procedure of Bjerrum et al. (4).

RESULTS

Previous attempts in our laboratory to use RIEP to quantitate RLV p30 have met with limited success. Though altering the pH of the electrophoresis system was found to change the IEP patterns obtained, the resulting immunoprecipitates were not sharply delineated and appeared to have been formed more from diffusion than by electrophoresis.

After an observation in our laboratory that p30 antigenic determinants are still able to react in immunodiffusion with specific antiserum after 1 min of boiling in 1% SDS, we reasoned that SDS might facilitate RIEP of p30 by conferring an artificial electrophoretic mobility on the polypeptide. An experiment was therefore outlined to test this hypothesis.

Figure 1 shows the effect of treating RLV samples with increasing amounts of SDS on the relative electrophoretic mobility of RLV p30. Though most of the untreated RLV p30 displayed a slight cathodal electrophoretic mobility, samples treated with increasing amounts of SDS displayed increasing anodal electrophoretic mobilities. In addition to reversing and greatly increasing the electrophoretic mobility of RLV p30, the anionic detergent also changed p30's immunoprecipitation pattern. Untreated RLV lysates usually yield rather broad p30 immunoprecipitates with clear indication of two electrophoretically distinct populations possessing identical p30 antigenic determinants (10, 17). However, SDS treatment minimized these differences and yielded a population of electrophoretically homogeneous antigenic determinants. Treatment with 5% SDS per mg of RLV protein conferred an electrophoretic mobility on the p30 molecule approximately equivalent to that of native bovine serum albumin.

RIEP was subsequently attempted with SDS-treated lysates (Fig. 2). The results showed that SDS concentrations between 0.5 and 2.0%/mg of RLV protein conferred an increased anodal electrophoretic mobility on RLV p30 and resulted in the formation of sharp, clearly delineated rocket immunoprecipitates. Smaller, poorly defined immunoprecipitates were obtained when the SDS concentration was lower than 0.5%/mg of RLV protein. Because of these results, we have adopted 0.5% SDS per mg of RLV protein for routine RIEP assay of RLV p30.

To substantiate that rocket immunoprecipitates were due to an immunoprecipitation reaction between RLV p30 and its specific antibody, the above samples were simultaneously electrophoresed into an agarose gel containing 5% normal goat serum (Fig. 2). Immunoprecipitates were not obtained with normal goat serum agarose, thereby ruling out the possibility that immunoprecipitates were due to the nonspecific reaction of SDS with normal serum components (5).

The effect of SDS on the reactivity of RLV

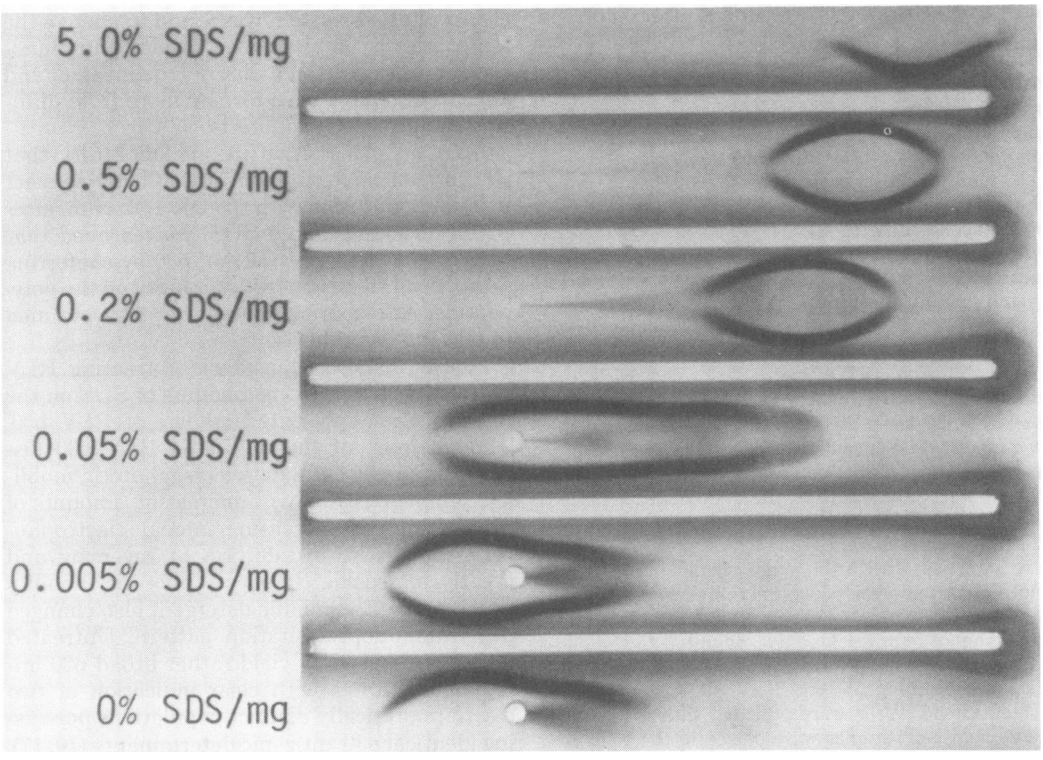

FIG. 1. *IEP of SDS-treated RLV lysates. After treatment with various concentrations of SDS, 5-μl volumes of RLV (10 μg of total protein) were subjected to electrophoresis until bromophenol blue tracking dye migrated to the end of the troughs at the anode (right). Anti-RLV p30 serum was placed in the troughs and allowed to incubate at 37°C for 24 h.*

p30 antigenic determinants was investigated to verify that the detergent does not partially destroy or otherwise reduce the polypeptide's ability to react with its specific antibody. For these investigations, RID (which relies upon diffusion rather than upon electrophoresis to form immunoprecipitates) was used. An RLV lysate was divided into portions and either treated with various concentrations of SDS or carbamylated for 3 h at 37°C with potassium cyanate (4). An additional untreated portion served as a control. When serial twofold dilutions of the preparations were assayed by RID (Fig. 3), no differences were observed between the ring sizes of the immunoprecipitates of the SDS-treated RLV lysates, the carbamylated RLV lysate, or the control RLV lysate. In addition, no precipitation was observed (either before or after staining) around wells receiving serial twofold dilutions of a 2% solution of SDS. These results indicate that the SDS treatment neither destroys p30 antigenic determinants nor impairs formation of p30 immune complexes.

The effect of detergent treatment on the quantitative aspects of RIEP assay was also investigated. Two portions of an RLV preparation were carbamylated for 3 and 18 h, respectively; and portions of each preparation were then treated with 0.5% SDS per mg of RLV protein. The four preparations were co-electrophoresed into a 2% agarose gel containing 5% anti-RLV p30 serum (Fig. 4). All RLV samples formed rocket immunoprecipitates upon electrophoresis, indicating that carbamylation of RLV alone is sufficient to alter the electrophoretic mobility of p30 enough to permit RIEP analysis and quantitation. The rockets formed by the SDS-treated samples were approximately 1.2 and 1.1 times higher than their corresponding 3-h and 18-h carbamylated RLV controls. However, this difference is not interpreted as being significant, since the rockets of the SDS-treated samples were visible at an earlier time. Since the electrophoretic mobility of SDS-treated RLV p30 was greater than carbamylated p30, the slightly larger rockets of SDS-treated samples were due to faster electrophoresis. This conclusion is further supported by the fact that the 18-h carbamylated RLV preparation formed a rocket 1.3 times higher than the 3-h carbamylated prepa-

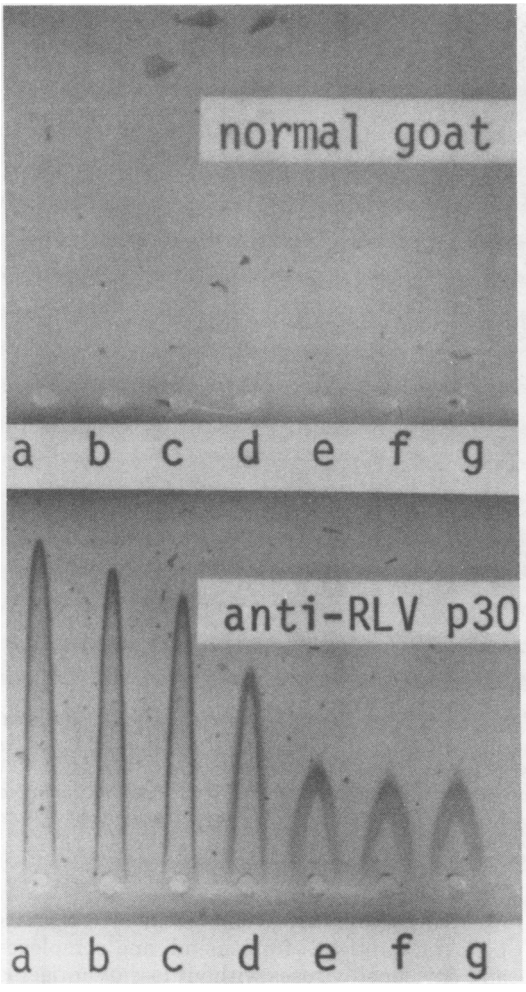

FIG. 2. *RIEP of SDS-treated RLV lysates. After treatment with various concentrations of SDS, 2-μl volumes of RLV (2 μg of total protein) were simultaneously electrophoresed into 2% agarose containing either 5% normal goat serum (top) or 5% goat anti-RLV p30 serum (bottom). SDS concentrations used were: (a) 2%, (b) 1%, (c) 0.5%, (d) 0.2%, (e) 0.05%, (f) 0.005%, and (g) 0% per mg of RLV protein.*

ration in the time allotted for the electrophoresis.

Inclusion of 0.5% SDS per mg of RLV protein was sufficient to allow RIEP quantitation of RLV p30 and was therefore adopted for routine RIEP assay of RLV samples. Figure 5 shows the results of one such analysis in which an estimation was made of the amount of "viral protein" in fractions collected from a model K-II zonal ultracentrifuge after initial concentration of 118 liters of RLV material. When plotted (Fig. 6), the rocket heights given by the RLV standards yielded a curvilinear plot between 125 and 4,000 ng of total RLV protein. The nonlinearity of the standard plot is attributable to incomplete electrophoresis. Because purified RLV preparations were used to make the standards, quantitation must be made in terms of viral protein. Other assays performed on the fractions identified by RIEP as containing p30 antigens (e.g., virus particle counts by electron microscopy, ribonucleic acid-directed deoxyribonucleic acid polymerase, total protein, and viral infectivity) verified that the QIEP technique had not only qualitatively identified the fractions that contained RLV, but had also provided a quantitative estimate of the amount of virus in each fraction (data not shown).

DISCUSSION

The binding of the anionic detergent SDS to polypeptides and other biological macromolecules has been studied in detail (20–22). Its usefulness in conjunction with polyacrylamide gel electrophoretic techniques both for solubilization and for obtaining estimates of molecular weights is well established (23, 26). However, the use of SDS with immunological techniques has generally been discouraged in the literature.

Several reports have been made of SDS causing nonspecific precipitation of normal serum proteins (especially 7S immunoglobulin) during agar gel diffusion and IEP of SDS solutions (5, 11, 19). In contrast, Crumpton and Parkhouse (8) reported that precipitation of antigen-antibody complexes was markedly inhibited by 0.5% SDS. It is therefore evident that care must be exercised when using SDS in immunoprecipitation techniques, as it may yield false-positive or false-negative immunoprecipitation data.

The anomalous effects that SDS can elicit have also been observed in QIEP techniques. Nielsen and Bjerrum (16) reported that 1 to 2 mmol of SDS in a 5-μl sample was sufficient to cause disturbances in the normal precipitation patterns obtained upon QIEP analysis of either human serum proteins or membrane proteins from bovine milk fat globules. The disturbances included loss of certain immunoprecipitates, the appearance of new immunoprecipitates, and the apparent non-immunological precipitation of other serum proteins. Interestingly, these effects could be partially overcome by incorporating nonionic detergents in either the samples or the agar gels before QIEP analysis.

Whereas there is no doubt that the strong conformation changes and alteration of charge induced by SDS can precipitate certain polypeptides and/or interfere with their specific immunological reactivity, SDS has, nonetheless, been

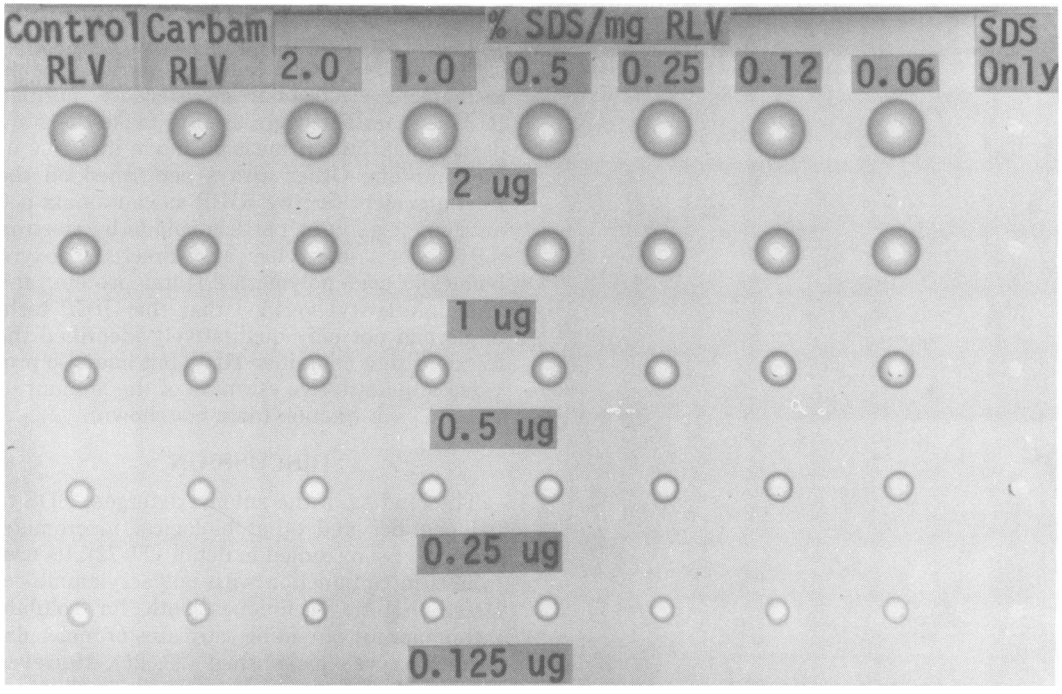

FIG. 3. *RID of SDS-treated and carbamylated RLV lysates. After treatment either with various concentrations of SDS or by carbamylation with potassium cyanate, serial twofold dilutions were made of RLV lysates containing 1 mg of total protein per ml. Two-microliter volumes of each dilution were placed in wells in 2% agarose containing 5% anti-RLV p30 serum and allowed to incubate for 24 h at 37°C. Controls included serial twofold dilutions of an untreated RLV lysate and a 2% SDS solution. SDS concentrations used were: 2%, 1%, 0.5%, 0.25%, and 0.6% SDS per mg of RLV protein.*

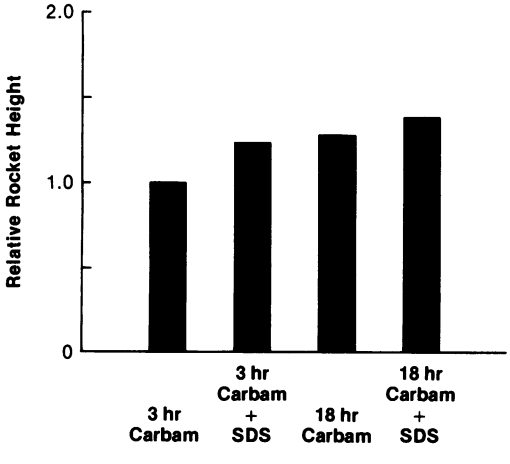

FIG. 4. *Influence of SDS on RIEP of carbamylated RLV preparations. After carbamylations for either 3 or 18 h, portions of identical RLV preparations were treated with 0.5% SDS per ml of RLV protein and analyzed by RIEP assay for RLV p30. Resulting heights of rocket immunoprecipitates are plotted relative to the untreated 3-h-carbamylated control.*

used successfully by a number of investigators to extract antigens from membrane complexes and enveloped viruses without loss of antigenic reactivity (7, 9, 10, 18). In these studies, the investigators used the anionic detergent solely for extraction purposes and generally removed it by cold precipitation, KCl precipitation, or ion-exchange chromatography (25) before performing immunological characterization of the extracts.

Investigators studying uromucoid have used the dissociating properties of SDS to facilitate a variety of immunological techniques (11, 15). Because native uromucoid possesses a molecular weight of 7×10^6, the large glycoprotein molecule must first be dissociated into its 80,000-dalton subunits before analysis by agar gel immunodiffusion and IEP is possible (11). The investigators titrated the SDS and determined that 0.3% SDS was sufficient to maintain the uromucoid in its subunit state, thereby allowing RID and RIEP analyses to be performed. Higher concentrations of SDS interfered with both assays by causing a nonspecific precipitation in RID assay and

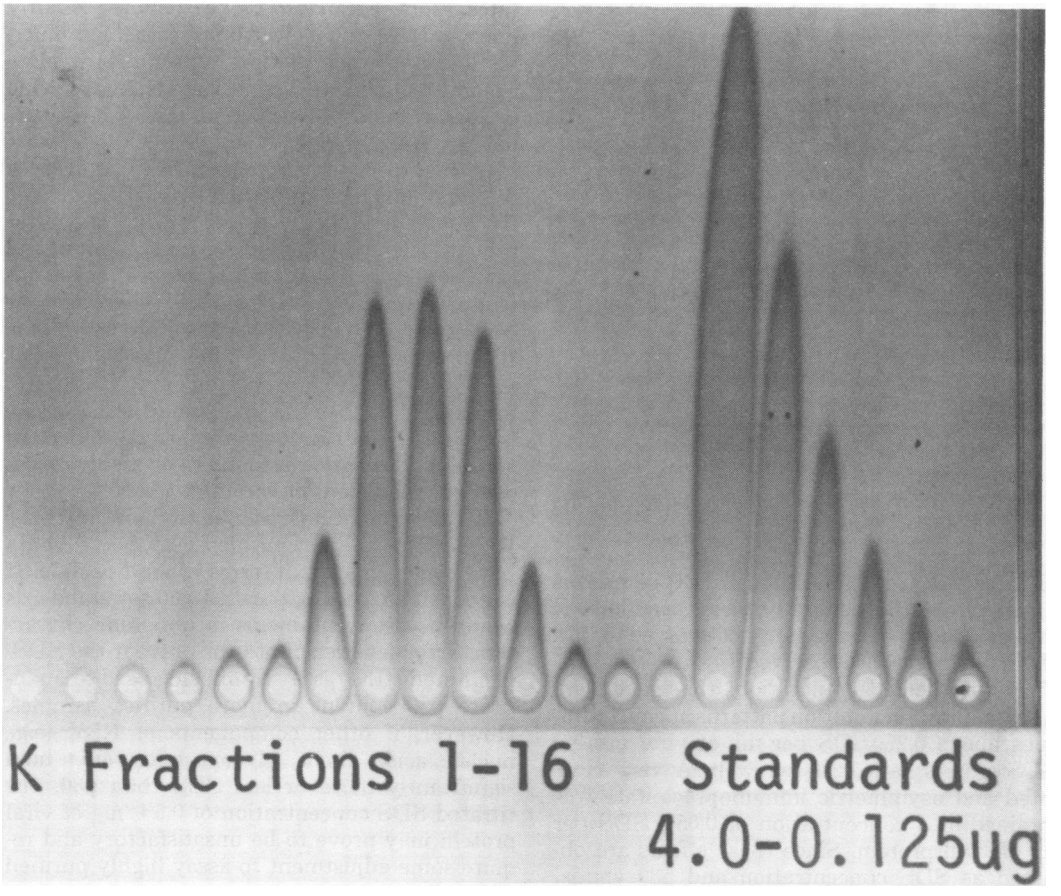

FIG. 5. *RIEP quantitation of viral protein in zonal ultracentrifuge gradient fractions after concentration of 118 liters of RLV material. Sucrose was removed by differential ultracentrifugation, and portions of each fraction were treated with 0.5% SDS per mg of protein. Two-microliter volumes of the samples and serial twofold dilutions of a standard purified RLV preparation containing 2 mg of protein per ml and 1.0% SDS were analyzed by RIEP assay for RLV p30.*

yielded artifactual overestimations of uromucoid concentration in RIEP assay.

The work presented here also describes the use of SDS in IEP techniques, but for a different purpose: to noncovalently confer an increased anodal electrophoretic mobility on antigens that otherwise migrate too slowly during conventional electrophoresis to permit QIEP analysis. The data presented (Fig. 1) show that treatment of RLV lysates with the anionic detergent SDS at concentrations between 0.2 and 2.0% SDS per mg of viral protein (SDS:protein ratio, 2:1 to 20:1 [wt/wt]) greatly increases the anodal electrophoretic mobility of RLV p30. The treatment does not cause a loss of antigenicity (as determined by RID quantitation, Fig. 3) and does not cause nonspecific precipitation of serum proteins during RID, IEP, or RIEP analysis (Fig. 1 through 3).

As mentioned above, other investigators have found that SDS can cause nonspecific precipitation of normal serum proteins or interfere with specific immunological precipitation reactions. We did not observe these effects during our studies. Though the reasons for this could be many, we attribute it to binding of the anionic detergent by RLV components, possibly to a counter-effect exhibited by the two nonionic detergents (Nonidet P-40 and deoxycholate) present in the RLV lysates (16), or to the antigen-antibody system under investigation.

SDS treatment of RLV preparations was found to have an additional effect on the immunoprecipitation pattern of RLV p30 during

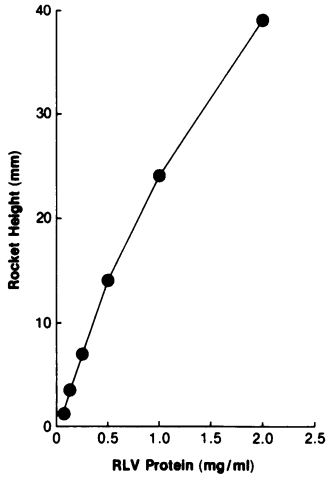

FIG. 6. *Plot of rocket immunoprecipitate heights of RLV standards shown in Fig. 5.*

IEP analysis (Fig. 1). Although IEP of murine oncornavirus p30 generally yields asymmetric patterns due to heterogeneity of charge (10, 17), SDS treatment was observed to overcome this effect and yielded preparations that gave symmetrical p30 precipitation patterns at concentrations above 0.2% SDS per mg of viral protein (SDS:protein ratio, 2:1 [wt/wt]). A very elongated and asymmetric immunoprecipitate was obtained at a concentration of 0.05% SDS per mg of viral protein. Since these effects are obtained as SDS concentration and p30 anodal electrophoretic mobility increase, they are attributed to increased binding of SDS by the p30 molecule and an obscuring of native charge heterogeneity.

To verify that treatment with SDS does not cause abnormally high rocket immunoprecipitates (3), an RLV sample was carbamylated and compared by both RID and RIEP assay before and after SDS treatment. Both RID and RIEP analyses (Fig. 3 and 4) indicated that SDS treatment does not cause artifactual rocket heights.

At SDS concentrations of 0.5%/mg of viral protein (SDS:protein ratio, 5:1), RLV p30 possesses sufficient electrophoretic mobility to yield sharp, clear rocket immunoprecipitates in RIEP analysis. This concentration was therefore adopted as a standard SDS concentration at which to assay unknown samples for p30 content. This assay has been used in our laboratories to screen gradient fractions (as presented in Fig. 5), cell lysates, and virus concentrates (both crude and highly purified) for the presence of RLV p30 antigenic determinants. In all materials tested to date, RLV p30 (when present) has yielded sharp, clear rocket immunoprecipitates. Plots of standard rocket heights are fairly linear (Fig. 6), and if electrophoresis is continued for longer periods of time, the standard plots become truly linear. To our knowledge (24), this is the first report of a QIEP assay for an oncornavirus antigen.

In the experiments reported, RIEP in agarose gels containing 5% antiserum was used to detect RLV p30 in as little as 125 ng of total viral protein. Reducing the antiserum content of the agarose results in larger, less intense rocket immunoprecipitates. For instance, when agarose containing 3.3% anti-RLV p30 serum is used for RIEP, 3-mm rockets are obtained with 30 ng of total RLV protein (unreported data). It is therefore apparent that SDS-facilitated RIEP assay is a sensitive assay for detecting RLV p30. In addition, the assay was found to be highly reproducible (coefficient of variability, <3.0%).

Because the standards used in this study were RLV lysates and not purified RLV p30, all data have been reported in terms of viral protein. It is not felt that using purified p30 for standards would pose any problems in obtaining characteristic rocket precipitates or prevent the SDS-facilitated RIEP assay from being used to quantitate p30 content in more purified samples. However, if other components of RLV (e.g., nucleic acids, lipids, glycoproteins, etc.) bind significantly more or less SDS than p30, our titrated SDS concentration of 0.5%/mg of viral protein may prove to be unsatisfactory and require some adjustment to assay highly purified p30.

The data presented were obtained on a single antigen-antibody system. Because SDS binding is a general property of proteins, we speculate that SDS can be used in similar manner with other antigen-antibody systems. Indeed, we have already successfully applied the method to detect antigens of other murine and primate oncornaviruses.

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