Non-Immunological Precipitation of Serum by Sodium Dodecyl Sulfate in Agar Diffusion

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Marmoset serum or serum of other species of animal may react with sodium dodecyl sulfate and forms nonspecific precipitin lines in agar diffusion. The protein detergent complexes are not readily dialyzable. Therefore precipitin lines derived from studies that use sodium dodecyl sulfate-treated antigens in agar diffusion must be interpreted with caution.

Sodium dodecyl sulfate (SDS) has been used with considerable success to dissociate viral particles in studying their structural proteins (4, 7). Non-immunological precipitation of SDS and serum proteins in agar diffusion has recently been reported (9). The precipitation may be misinterpreted as antigen-antibody reactions. This report presents another example of nonspecific precipitation between SDS and serum of marmoset and of other animals. To our knowledge, the unique precipitin reaction between marmoset serum and SDS has not been described previously.

MATERIALS AND METHODS

Sera. Marmoset (Oedipomodas oedipus) sera were collected from our previous studies (1). Other sera were randomly selected from humans, monkeys, mice, horses, and rabbits.

SDS. A stock solution of 98% purity of SDS was obtained from Proctor and Gamble, Cincinnati, Ohio. Twofold dilutions of this solution were prepared separately in distilled water and in phosphate-buffered saline (PBS).

Immunodiffusion. Double gel diffusion method of Ouchterlony was used (8). The supporting agar gel consisted of 0.8% agarose (Sigma Chemical Co., St. Louis, Mo.) in 0.01 M PBS (pH 7.2) with 0.15 M NaCl and 0.01% merthiolate. Serum treated with various concentrations (0.9, 1.8, 3.6, 7.2, and 14.5%) of SDS (1:1 mixture) or diluted SDS solution alone was loaded in the central well, serum specimens were placed in the peripheral wells. The agar plates were incubated at 4°C in a moist chamber for 48 h. Photography was done over direct light without any staining or modifications.

Dialysis. A 20-ml amount of a pool of marmoset sera was equally divided into four portions. Two of these portions were treated with 1 ml of 7% SDS. Each of these treated and untreated sera was dialyzed separately against PBS (pH 7.2) and distilled water (pH 7.0) at 4°C. The dialyzing solution was changed once daily for nine consecutive days. Each day 0.5 ml of sera was removed from the bag and it was kept at 4°C until further testing. In double gel diffusion test, these dialyzed preparations were placed in peripheral wells, and the original pool of marmoset sera was placed in the central well.

RESULTS

Precipitin lines formed by SDS and marmoset serum. SDS at a concentration of 1.8% or greater when reacted with SDS-treated or untreated marmoset sera consistently formed precipitin lines. Such precipitin lines were easily identified when SDS was placed in the center well and sera placed in peripheral wells (Fig. 1 and 2). These precipitin lines are usually formed within a few hours and they reached completion in 24 h. In contrast to other animal sera, marmoset sera invariably produced distinctive and sharp lines. More than one precipitin band were seen in some marmoset sera (Fig. 1c and e).

Precipitin lines formed by SDS and other animal serum. SDS also formed non-specific precipitin lines with sera of human, rhesus monkey, horse, rabbit, and mouse (Fig. 2 and Table 1). However, the precipitin lines were less distinctive. It was also noted that such precipitin lines were not observed in some of the specimens even after repeated testing (Table 1).

Effects of SDS concentrations and diluents on precipitations. Precipitation developed at the concentrations of 1.8% or greater. No precipitation was observed at the concentrations of less than 0.9% of SDS. Either distilled water, PBS, or marmoset serum used as diluent did not alter the formation of the precipitates. Recently it has been found that SDS nonspecifi-
cally precipitate proteins and lipid components of whole serum of guinea pig, rabbit, and human (9). Conditions required for the precipitation of proteins by SDS and the properties of the protein-detergent complexes have been studied in detail (6, 9, 10).

**Effects of dialysis on formation of precipitin lines.** Dialysis of pooled marmoset sera, either treated or untreated with SDS, against PBS or distilled water did not prevent the formation of precipitates. There was only a slight reduction of intensity of precipitin formation after one day of dialysis; however, further reduction of reactivity over a period of 9 days of dialysis was not observed.

**DISCUSSION**

Non-immunological precipitin formation in double gel diffusion has been briefly reviewed by Gardner et al. (2). Notable examples of nonspecific precipitation occurred in red cell hemolysate and serum from the same subject, serum protein and gamma-glutamyl polypeptide, concanavalin and polysaccharide or serum glycoproteins, cell wall protein of staphylococcus aureus and gamma globulin of human and

FIG. 1. Agar double-diffusion of SDS and marmoset sera. The center well contained 7.0% SDS in PBS. The peripheral wells contained marmoset serum: a, #315; b, pooled sera; c, #307; d, #312; e, #322.
FIG. 2. Agar double-diffusion of SDS and animal sera. The center well contained 7.0% SDS in PBS. The peripheral wells contained serum from various sources: a, marmoset #315; b, horse; c, human; d, rabbit; e, rhesus monkey.

TABLE 1. Precipitin formation between SDS and sera from various species of animals

| Serum    | No. positive/ no. tested | % Positive | Intensity of precipitin* |
|----------|--------------------------|------------|-------------------------|
| Marmoset | 150/150                  | 100        | +++                     |
| Horse    | 20/20                    | 100        | +                       |
| Human    | 73/80                    | 91         | +                       |
| Monkey   | 8/10                     | 80         | +                       |
| Rabbit   | 36/40                    | 90         | +                       |
| Mice     | 43/50                    | 86         | +                       |

* Intensity of precipitin formation: +++ = strong, ++ = moderate, + = weak.

guinea pig, serum and gut tissue from the same animal, dextran sulfate and beta-lipoproteins of whole serum, etc. (2, 5). Reasons for the occurrence of such nonspecific precipitation and its physiological significance are not well understood. Many considered these reactions as undefined chemical interactions (2).

Our studies confirmed the findings of an earlier report (9) in regard to precipitin line formation between SDS and sera from various species of animals. In addition, we have further demonstrated that such reactions also occur in sera of nonhuman primates (marmoset and rhesus monkey). Marmoset sera, in contrast to sera of human, rhesus monkey, and other animals, formed very heavy precipitin lines when reacted with SDS. Biochemical findings of marmoset serum have demonstrated some differences from those of human sera (3).

SDS is widely used to disrupt virions for the study of proteins of many viruses. The results derived from studies that use SDS-treated anti-
gens in agar diffusion must be interpreted with caution, particularly when high concentrations of SDS are used (7), and since these protein-detergent complexes are not readily dialyzable.

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