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SAPONIN ADJUVANTS

V. PRECIPITATION OF SERUM COMPONENTS BY NON-PURIFIED SAPONIN ADJUVANTS IN AGAR GEL DIFFUSION

By
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DALSGAARD, K.: *Saponin adjuvants. V. Precipitation of serum components by non-purified saponin adjuvants in agar gel diffusion.* Acta vet. scand. 1977, *18*, 361—366. — The immunologically active adjuvant Quil A does not induce precipitating antibodies in rabbits. This was tested by immunodiffusion of serum samples taken after repeated injections of Quil A. Quil A does not react non-specifically with any of 6 different animal sera tested (rabbit, guinea pig, horse, sheep, cattle, and pig). Two commercially available saponins with known adjuvant activity (Saponin MT, E. Merck, and Saponin P 3, Food Industries) produced non-specific precipitation in double gel diffusion tests with all the sera, as did crude extracts of the South-American tree Quillaja saponaria Molina.

This phenomenon in relation to the local tissue damage caused by non-purified saponin preparations is discussed.

adjuvant; saponin; gel precipitation; immunodiffusion; Quil A.

Some commercially available saponins have adjuvant activity of great practical importance to the vaccination of cattle against foot-and-mouth disease (FMD) (*Charlier et al.* 1973). In previous reports (*Dalsgaard* 1972) concerning the standardization of these natural compounds a virtually pure substance, Quil A, with adjuvant activity was isolated. During experiments in rabbits undertaken to estimate whether this substance is in itself immunogenic, it was discovered that non-purified saponins produced non-specific precipitation in agar gel diffusion tests. This precipitation did not only occur with rabbit serum but also with all other animal sera tested. The present report describes these phenomena.

MATERIALS AND METHODS

Sera

Rabbit, guinea pig, horse, sheep, cattle and pig sera were randomly selected from laboratory animals at this institute.

Saponins

Two commercially available saponins were used, Saponin gereinigt MT (E. Merck, Darmstadt, W. G. lot No. 6272202) and Saponin P 3 (Food Industries, Cheshire, U. K. lot No. 6202034).

Crude and dialysed extracts of Quillaja saponaria Molina were prepared as described previously (*Dalsgaard* 1970). Quil A was isolated as described elsewhere (*Dalsgaard* 1974).

Immunization

The immunization procedure of Harboe and Ingild (*Axelsen et al.* 1973) was followed. Quil A was dissolved in saline to a concentration of 10 mg/ml. This solution was mixed with an equal volume of Freund's incomplete adjuvant, and 100 µl doses were injected into 5 albino rabbits bred at this institute. The injection was given in the thicker part of the skin above the scapula, and the dose was placed as superficially in the skin as possible. Injections were given on day 0, 14, 28 and 42. On day 50 blood samples were taken from an ear vein, and each sample was tested individually for precipitating antibodies against Quil A by immunodiffusion.

Immunodiffusion

The double gel diffusion method was used (*Ouchterlony* 1958). The supporting gel consisted of 1.0 % agarose (Litex, Glostrup, Denmark) in 0.01 M-PBS pH 7.2 with 0.15 M-NaCl and 15 mM-NaN_3. Serum specimens diluted 1:10 in the supporting buffer were placed in the peripheral wells, and the various saponin solutions (2 % in supporting buffer) were placed in the central well. The agarose plates were incubated in a moist chamber for 48 hrs. Photography was done over direct light without any staining or further enhancement of the precipitin lines.

RESULTS

Immunization experiments

The sera of the 5 rabbits immunized with Quil A showed no precipitating antibodies in the double gel diffusion test, and no

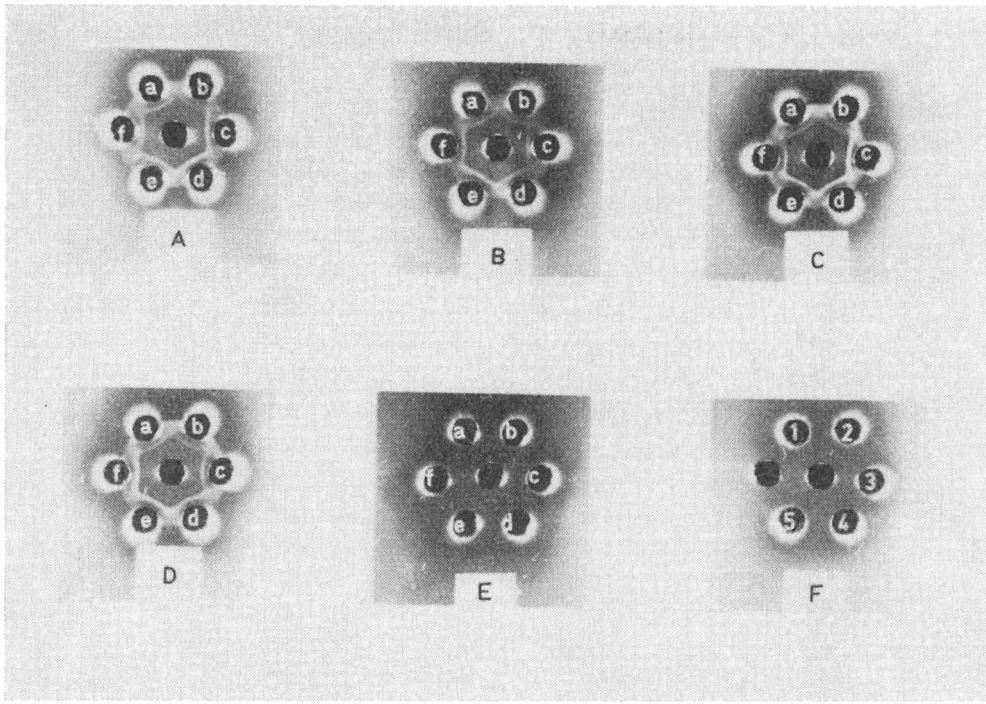

Figure 1. Double immunodiffusion of different saponin preparations (central wells) against various animal sera (peripheral wells): a. rabbit; b. guinea pig; c. horse; d. sheep; e. cattle; f. pig serum, all diluted 1:10. 1—5: sera of rabbits immunized with Quil A. A. Saponin "gereinigt". B. Saponin "P 3". C. Crude quillaja extract. D. Dialysed quillaja extract. E and F. Quil A.

non-specific precipitation of serum by Quil A was seen (Fig. 1 F and E, respectively).

Precipitin lines formed by commercially available saponins

Two % solution of the 2 commercially available saponins tested formed precipitin lines with all the animal sera. The results are shown in Fig. 1 A and B. Such precipitin lines were easily detectable within a few hours of diffusion and they reached maximum within 24 hrs.

Precipitin lines formed by extracts of Quillaja saponaria Molina

Aqueous extracts of the cortex of the South-American tree Quillaja saponaria Molina have been demonstrated to possess

adjuvant activity (*Dalsgaard* 1972), and the saponin adjuvant Quil A has been isolated from this material (*Dalsgaard* 1974). Experiments were set up to investigate the ability of these materials to form non-specific precipitin lines on different stages of purification. Three specimens were applied: 1) the crude aqueous extract of the cortex, 2) the crude extract after dialysis, 3) Quil A, the saponin adjuvant isolated from the dialysed extract. It can be seen in Fig. 1 C and D that as well the crude extract as the dialysed extract produced precipitin lines with rabbit, guinea pig, horse, sheep, cattle and pig sera. The intensity and the velocity of the formation of these lines were the same with these 2 materials indicating that the precipitating agent(s) could not be removed by dialysis. Quil A (Fig. 1 E) showed no precipitin lines, indicating that these lines are not produced by the adjuvant active substance, but rather by some accompanying substance(s) in non-purified material. Quil A was tested in the same system in concentrations up to 10 % without producing any precipitin lines (not shown).

DISCUSSION

Saponins are often included in the vaccines used to immunize cattle against FMD. Commercially available saponins are extracted from different plant species (*Basu & Rastogi* 1967), but the immunologically active saponins seem to be derived from Quillaja saponaria Molina (*Dalsgaard* 1970). The 2 commercial saponins used in this report were also derived from this tree. They were identified using a thin-layer chromatographic method published elsewhere (*Dalsgaard* 1970). The major drawback of using commercially available saponins is that some of them produce severe tissue damage at the site of injection, when administered in doses sufficient to enhance the immune response (*Charlier et al.* 1973). In a preceding publication (*Dalsgaard* 1974) it was demonstrated that a purified substance, Quil A, produces only a minor local reaction in therapeutic doses, suggesting that part of the reaction produced by non-purified saponins is due to other substance(s) than the immunologically active one(s). In the present paper evidence is presented that some of the impurities of saponin adjuvants have serum precipitating properties. Saponins are surface active agents, and non-specific precipitation of serum by other surface active agents

such as SDS has been reported by others (*Cho & Feng* 1974). Quil A, however, being in itself a negatively charged surface active agent (*Dalsgaard* 1974), did not form precipitin lines with animal sera. The nature of the impurities producing this precipitation has not been investigated, but it is suggested that the serum precipitation produced by these impurities may be one of the mechanisms of their local reactivity. Extracts from saponin-containing plants are widely used in industry because of their ability to emulsify and clean. Of special importance is their application in cosmetology for the production of certain shampoos. Unfortunately these shampoos cause local skin reactions in certain persons. In a recent patent (Belg. pat. 778.388) it has been claimed that the local reactivity can be diminished by the precipitation of the crude extracts with egg albumin. This finding is in agreement with our results that crude extracts of Quillaja saponaria Molina have protein precipitating properties.

Due to their detergent activity saponins are frequently used for the disruption of biological membranes (*Schneider et al.* 1971, *Arstilla* 1974). The results of the present paper indicate that if such preparations are used for agar gel diffusion studies, the plates must be interpreted with caution.

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SAMMENDRAG

Saponin adjuvanter. V. Urensede saponin adjuvanter præcipiterer serumkomponenter i agar gel diffusion.

Den immunologisk aktive adjuvant Quil A inducerer ikke præcipiterende antistoffer i kaniner. Dette blev undersøgt ved immunodiffusion af serumprøver udtaget efter gentagne injektioner med Quil A. Quil A reagerer ikke uspecifikt med 6 forskellige animalske sera (kanin, marsvin, hest, får, kvæg og svin). To kommercielt tilgængelige saponiner med kendt adjuvant aktivitet (Saponin MT, E. Merck og Saponin P 3, Food Industries) gav uspecifikke præcipitationer i immunodiffusion med alle disse sera. Rå ekstrakter af det sydamerikanske træ Quillaja saponaria Molina gav også denne reaktion. Dette fænomen i forbindelse med den lokale vævsreaktion, der forårsages af urensede saponiner, diskuteres.

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