Vol. 114, No. 2, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 29, 1983 Pages 699-704

ASSAY OF DETERGENTS BY ROCKET ELECTROPHORESIS IN AGAROSE GELS CONTAINING RED BLOOD CELLS: "ROCKET HEMOLYSIS"

B Sundquist[1], K Dalsgaard[2] and B Morein[1,3]

[1] National Veterinary Institute, S-750 07 Uppsala, Sweden

[2] State Veterinary Institute for Virus Research, Lindholm, DK-4771 Kalvehave, Denmark

[3] Department of Virology, Faculty of Veterinary Medicine, Biomedical Center, Box 585, S-751 23 Uppsala, Sweden

Received June 8, 1983

A method is described for quantitation of charged detergents using their hemolytic property in an electrophoresis assay in agarose gels containing red blood cells. After electrophoresis the zone of hemolysis is directly proportional to the concentration of detergent in the sample. Using this technique we have determined the smallest detectable concentration for the negatively charged detergents, sodium dodecyl sulfate (SDS) and Quil A to about 10µg/ml and 25µg/ml, respectively and the positively charged cetyltrimethylammonium bromide (CTAB) to about 10µg/ml.

Detergents are widely used for the solubilization of biological membrane constituents (1). In the immunological field membrane protein antigens may be released in active forms by the use of detergents. Lymphocyte antigens may be prepared in this way. But the most extensive use of different detergents has been in the preparation of enzymes and viral antigens. Detergents chemically belong to very different types of substances, the only common feature being their surface activity. Quantitative assays of detergents in biological mixtures are mostly performed by chemical detection. Also, when possible radioactive labeling of detergents may be used as a precise way of quantitation (2). It may be usefull however, to make use of the biological activity. A common characteristic of detergents is the ability to lyse red blood cells (RBC s). This property has been used only to a limited extent except in plant biochemistry, where hemolysis is generally used for the detection of saponin detergents (3).

In our work with the preparation of highly immunogenic structures from mem-

0006-291X/83 $1.50

brane proteins of viruses we needed a quantitative assay for the saponin adjuvant Quil A, and we are here describing an assay for Quil A and other detergents using "rocket hemolysis" in agrose gels.

MATERIALS AND METHODS

Chemicals - All chemicals were Merck analytical grade unless otherwise indicated. Quil A was prepared as previously described (4). Before electrophoresis the detergents were all dissolved in the electrophoresis buffer described below. The detergents used were: Quil A, cetyltrimethylammonium bromide (CTAB), and sodium dodecyl sulphate (SDS).
Sheep red blood cells (SRBC s) - Sheep blood cells was collected in isotonic citrate/glucose buffer. RBC s were washed 3 times in saline. Packed cells were kept at 5°C for up to one week.
Electrophoresis - The general equipment for electrophoresis was used. The gel used consisted of 1% agarose type HSB, Litex, Glostrup Denmark in a buffer having the following composition: Barbitone sodium 4.12 g, Barbitone 0.8 g, Glucose 43 g, sodium azide 0.2 g and redistilled water to make 1 liter. Gels were cast on 10x10 cm glass plates using 15 ml of agarose gel, melted and kept at 56°C. SRBC s ($1.5x10^6$) were suspended in the gel before casting, giving a final concentration of 15000 cells/cm^3 plate. Wells, 4 mm in diameter were punched in the gel, and 15 ul samples were used. Electrophoresis was carried out at 10 V/cm for 1 hour. After the run the plates were pressed once without rinsing by Whatman 3 M paper. The plates were dried in a hot air stream and stained with the crocein scarlet stain as described by Crowle and Cline (5).

RESULTS

Most experiments were made with Quil A and CTAB being negatively and positively charged detergents respectively. In Fig 1 these two detergents were run together on the same plate. It can be seen that the negatively charged detergent migrated towards the positive electrode and visa versa. The zones of hemolysis can be clearly seen after staining only. When concentrations of the detergents to be assayed were kept relatively low (Fig 2) the height of the rocket was close to be directly proportional to the concentration in the sample, but if higher concentrations were applied the shape of the rocket was no longer triangular because of the lateral diffusion. In general the area enclosed by the hemolysis zone was directly proportional to the concentration. A precise estimate of the area was obtained by placing the plate in a photographic enlarger, and projecting the image on paper which then was cut and weighed. With uniform paper and the same enlargement, arbitrary units of area were obtained for all hemolysis zones. In Fig 3 these arbitrary units were plotted against concentration for the

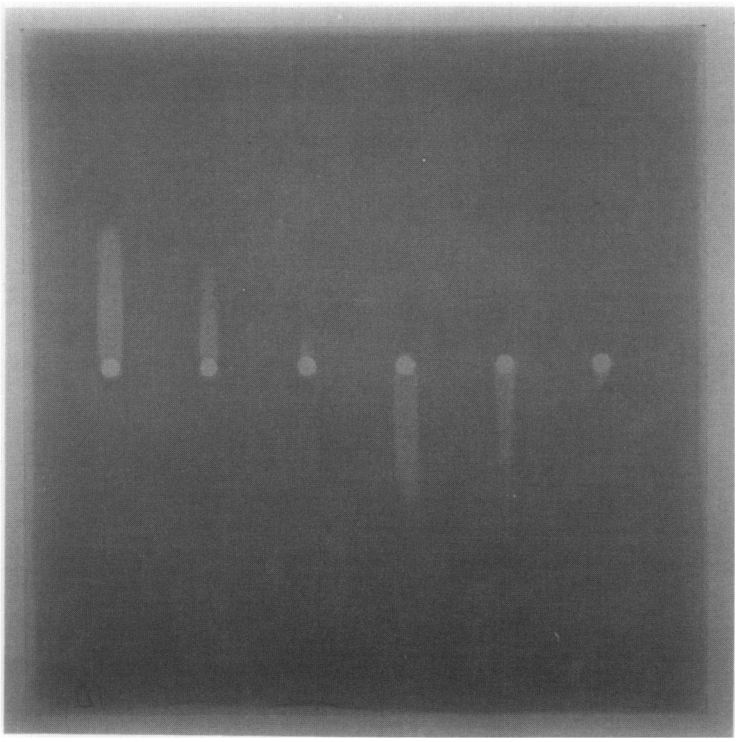

Figure 1. Rocket hemolysis in 1% agarose gel. Layer-thickness 1.5 mm, 4 mm wells. Gel contained 15000 SRBC s/cm^2. Electrophoresis at 10V/cm, 1h. Positive electrode at the top. Samples of 15 µl were applied. Left to right: Quil A, 200, 100 and 50 µg/ml; CTAB, 25, 12.5 and 6.25 µg/ml. Plate stained with crocein scarlet.

detergents Quil A, CTAB and SDS. The values were the mean of 3 estimations. Since the correlation lines were straight the sensitivity of the assay for a given detergent was read as being the concentration value where the line crosses the horizontal axis. For Quil A it can be seen that the smallest concentration detectable by the method was about 25 µg/ml.

DISCUSSION

The hemolytic property of detergents was used in a quantitative assay by electrophoresis in agarose gels containing red blood cells. Several parameters influencing the sensitivity of the assay was taken into consideration before the described procedure was finalized. It was considered that there might be differences in sensitivity to lysis of RBC s of different origin. But after having tested RBC s of several mammalian and avian species, we found no significant difference in sensitivity using rocket hemolysis. We

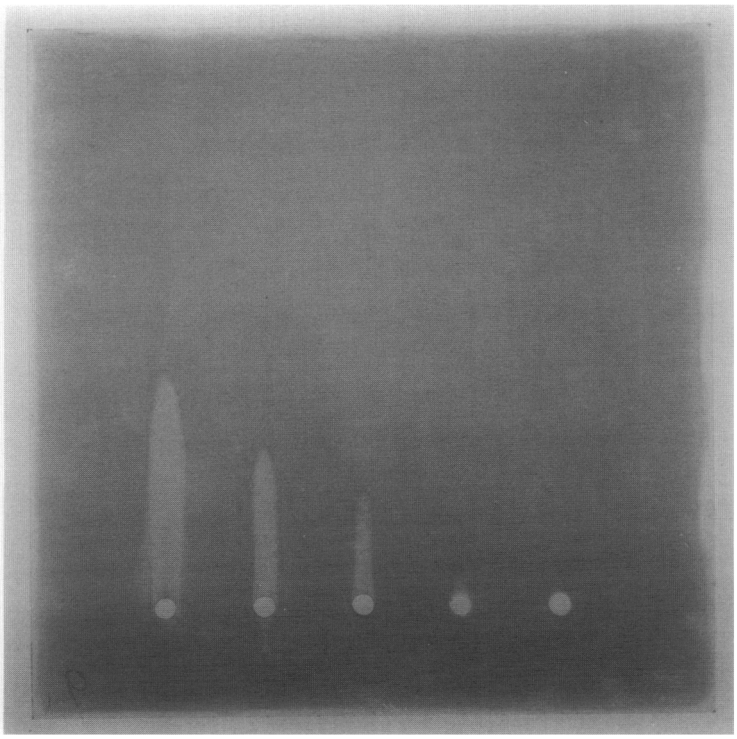

Figure 2. Rocket hemolysis in gel. Same conditions as in fig 1, but with negative electrode at the top. Left to right: CTAB, 50, 25, 12.5, 6.25 and 3.13 µg/ml.

therefore used sheep RBC s because they were readily available in the laboratory. However, more important was the concentration of RBC s in the agrose gel and we found that the lowest possible concentration of RBC s gave the highest sensitivity. The concentration used (15000 cells/cm^3) was chosen because it gave a reasonable contrast to the hemolysis zone after staining of the plate, whereas the cells could hardly be seen without staining. Lower concentrations were difficult to read and higher concentrations of RBC s decreased the sensitivity. It was considered to increase the sensitivity by priming the lysis of SRBC s by treating them with sub-lysing concentrations of the detergent to be assayed. But this did not seem to increase the sensitivity significantly, and it was omitted. Nor did slightly hypotonic electrophoresis buffers increase the sensitivity. Glucose was chosen to keep the isotonicity in a relatively low molar buffer because charged ions would produce excessive heat during electrophoresis. On

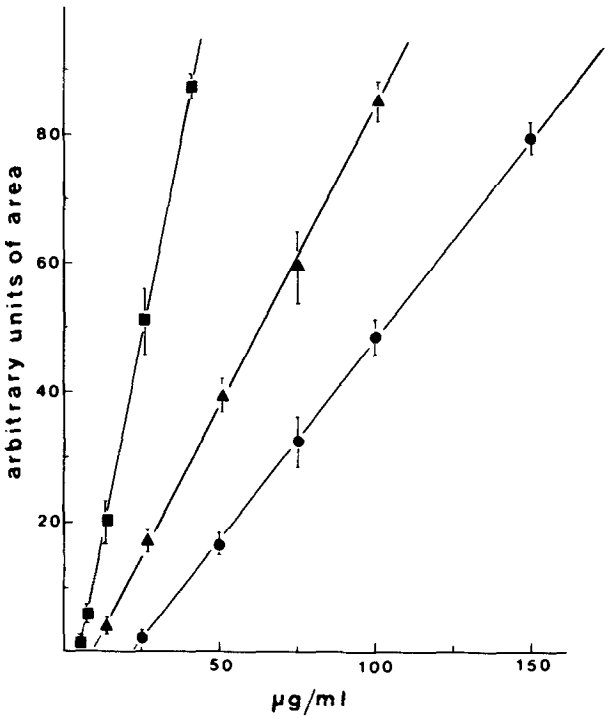

Figure 3. Rocket hemolysis of 3 detergents. Relationship between hemolysis area and sample concentration. ■——■ , CTAB; ▲——▲ , SDS; ●——● , Quil A. Mean value of 3 assays and vertical lines show standard deviations and dots represent individual values.

the other hand pH has to be stabilized, and we therefore used a regular barbitone electrophoresis buffer.

In the results only experiments with charged detergents are described, and the ideal gel was therefore an agarose of low electroendosmosis not interfering with the migration of the charged detergents in the electric field.

In some experiments (not shown) we also investigated the use of highly charged agarose gels for the rocket hemolysis of a non-ionic detergent (Triton X 100). The idea was that the electroendosmotic cathodic backflow would make the non-charged detergents migrate in the gel. This was indeed possible, but exact quantitation was difficult mainly due to the fact that our samples of Triton X 100 contained as well non-ionic negatively charged molecules!

We have used rocket hemolysis successfully for the quantitation of Quil A in viral vaccines where this substance was included as an adjuvant, and we conclude that the method is easy, fast and reliable.

ACKNOWLEDGEMENTS

We thank miss Helle Skøtt for expert technical assistance.

REFERENCES

1. Helenius, A. and Simons, K. (1975) Biochem. Biophys. Acta 415, 29-79.
2. Simons, K., Helenius, A. and Garoff, A. (1973) J.molec. Biol. 80, 119-133.
3. Stahl, E. Chromatographische und mikroskopische Analyse vor Drogen. G. Fischer, Stuttgart (1970).
4. Dalsgaard, K. (1974) Arch.f.gsmt.Virusforschung 44, 243-254.
5. Crowle, A.J. and Cline, L.J. (1977) J.Immun.Methods 17, 379-381