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GLYCEROL EFFICIENTLY LESSENS TOBACCO STREAK ILARVIRUS PARTICLES AGGREGATION DURING FORMALDEHYDE FIXATION

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We describe the use of glycerol to reduce aggregation of tobacco streak ilarvirus (TSV) particles caused by formaldehyde (FA) fixation. After treatment of partially purified TSV preparations with 1% FA in the presence of 10% glycerol, double radial immunodiffusion tests revealed the presence of two to four times more reacting virus than in preparations treated with 1% FA only. Consequently, the method provides higher quality preparations intended for different immunochemical purposes and biophysical and biochemical analyses. We expect it to be useful in fixation of viruses which tend to aggregate in the presence of FA.

Introduction

Tobacco streak ilarvirus (TSV) possesses a genome divided among several types of quasispherical particles (F r a n c k i et al. 1987). As the members of this group have labile particles it is necessary for many types of physico-chemical analysis, and for antiserum production, to fix purified preparations. For this purpose formaldehyde (FA) is mostly employed. FA fixation is traditionally used in the fixation of other labile plant viruses including cucumoviruses, bromoviruses, *etc.* FA preserves the structure of these viruses in conditions which otherwise lead to dissociation of the protein coat into subunits. During investigation of the effects of FA on some biophysical and biochemical properties of TSV particles, by analysis of their sedimentation and antigenic characteristics, we found that FA promotes particle aggregation, especially in highly

purified preparations; the degree of aggregation depended on different factors such as the concentration of FA, fixation duration, etc. (Krajačić 1989, Krajačić and Štefanac — unpublished results). Such aggregation does not occur with brome mosaic bromovirus (Richter et al. 1972). Here we show that the increased tendency of TSV particles to aggregate in the presence of FA can be considerably decreased by the use of glycerol.

Material and Methods

The Cle strain of TSV (Rana et al. 1987) was used. The virus was partially purified according to the procedure of Jones and Mayo (1973) for elm mottle virus modified by Rana et al. (1987). The particles were fixed in 1% FA (Krajačić 1989).

The degree of the virus aggregation was determined by double radial immunodiffusion tests using two antisera to TSV-Cle. One serum had a titre of 1/128 and the other 1/32 to the virus, and both had titres of 1/4 to normal plant proteins. The experiments were performed in 1% agar (Difco-Bacto) prepared in 0.01% Sörensen's buffer, pH 7.0 (standard buffer). In determining antigenicity of variously treated preparations two different methods were used. In one, initial virus preparations reacted with different dilutions of serum in saline, and the intensity of the precipitin lines was defined; in the other, various dilutions of the virus reacted with a constant serum concentration, and the virus titre was determined. The results were read after 24 h incubation in moist chamber at room temperature.

Results

First set of experiments

Partially purified preparation of TSV-Cle was mixed (1:1) with solutions of additives prepared in standard buffer, to give a final concentration of 5 mg/ml virus; control samples were mixed with buffer alone (Table 1). After storage for five months in the conditions shown in Table 1, the samples were examined by immunodiffusion tests.

In reactions of undiluted virus samples with undiluted serum (titre 1/128) the most intensive virus precipitin line developed with samples 1, 2 and 3; weak virus reaction was produced by sample 4, and in sample 5 it was completely absent (Fig. 1) regardless of the serum dilution used. The titre of sample 3 was one twofold dilution step lower than that of samples 1 and 2 which had an identical virus titre (*cf.* Table 1). In addition, virus precipitin lines of the sample conserved by glycerol only were sharper in comparison with lines of samples which included FA (cf. Fig. 1) no matter which virus dilution was used. The sample conserved, *i. e.* fixed, exclusively by adding FA produced particularly diffuse precipitin lines (Fig. 1).

The titre of host proteins of sample 1 was two dilution steps higher than for samples 2 and 3 (*cf.* Table 1). When compared with sample 1, which had no FA, the last two samples were also distinguished by more diffuse lines produced by host plant proteins (*cf.* Fig. 1).

Table 1. Effects of the mode of storage and fixation on partially purified TSV preparation as determined by virus and host protein titres in double diffusion serological tests*

Sample no.[a]	Aditives	Storage	Virus titre[b]	Host protein titre[b]
1	10% glycerol	frozen at −16 °C	32	8
2	10% glycerol and 1% FA	frozen at −16 °C	32	2
3	1% FA	4 °C	16	2
4	without additive (control)	4 °C	1	-
5	without additive (control)	frozen at −16 °C	0	-

*The assay was done after five month storage.
[a]Samples (virus conc. 5 mg/ml) were tested in triplicate.
[b]Virus and host protein titres (reciprocal values) determined by undiluted serum (titre 1/32).
FA = formaldehyde.
- not tested.

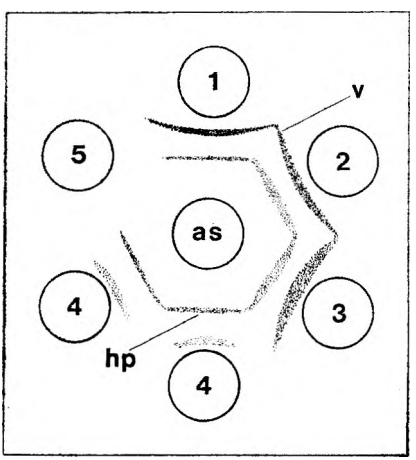

Fig. 1. Reaction of differently treated TSV samples (virus concentration 5 mg/ml) with TSV antiserum (as, titre 1/128) in double radial immunodiffusion. Samples are labeled as in Table 1. Virus precipitin reaction (v), host protein reaction (hp). The test was done after five months of storage.

Additional experiments

In the first additional experiment, two samples (now virus concentration 20 mg/ml) with additives as in samples 2 and 3, both kept under the same conditions (24 h at 4° C), were compared. The test showed that the sample which contained glycerol as well as FA gave a titre twice as high. This and the previous experiment show that much better quality preparations of TSV can be obtained by use of FA and glycerol during fixation.

In further experiments it was found that preparations in which glycerol was added before FA showed somewhat better antigenicity than those treated first with FA. The difference, however, was less than one twofold dilution step of the virus titre.

Discussion

The property of glycerol to protect the structure of protein molecules, particularly because it prevents ice crystal formation at low temperatures, is well known and, in connection with this quality, its universal utilization in preserving sera. Glycerol has also for a long time been added to virus preparations, especially of labile viruses, for the purpose of preserving particle structure and infectivity (Bercks 1950). This communication shows the usefulness of glycerol in decreasing the tendency of TSV particles to aggregate during FA fixation.

The TSV preparations containing 10% glycerol and 1% FA, kept at −16°C od 4°C, were more antigenic than samples with an equal amount of FA but no glycerol. This fact seems somehow surprising since FA fixation has been used for years with preparations of different viruses in order to preserve their antigenic strength (Van Regenmortel 1982). Consequently, if we wish to fix a preparation of TSV in order to employ it for different immunochemical experiments, it would be better not to do it in the way common so far. The procedure itself of virus conservation with glycerol only, is not adequate since this substance preserves virus particles only while the preparation is being kept at low temperatures. Such a preparation does not offen any advantages if the virus is intended for use in immunization, since the »native« virus particles will be intensely degraded in the experimental animal. In this case the joint use of glycerol and FA is to be preferred. As shown, the virus sample treated with 1% FA and 10% glycerol had the same virus titre as the one processed exclusively by glycerol and was fixed at the same time. We presume that what we have found for TSV is valid at least for some other members of the ilarvirus group, and also perhaps for the closely related alfalfa mosaic virus.

We suppose that the diffuse precipitin lines obtained in serological tests with FA-treated virus antigen and host proteins originated from the presence of small aggregates passing through the pores of gel, though not equally effectively as the free particles. The higher loss of host pro teins caused by fixation is in agreement with the fact that simple proteins aggregate more strongly under the action of FA (*cf.* Richter et al. 1972). As expected the tests of the samples kept at 4°C or −16°C without any additive yielded poor results.

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SAŽETAK

GLICEROL USPJEŠNO UMANJUJE AGREGIRANJE ČESTICA ILARVIRUSA CRTIČAVOSTI DUHANA PRI NJIHOVU FIKSIRANJU FORMALDEHIDOM

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Opisuje se upotreba glicerola radi smanjivanja intenziteta pojave agregiranja čestica ilarvirusa crtičavosti duhana (TSV), koju uzrokuje fiksiranje formaldehidom (FA). Nakon obrade djelomično purificiranih preparata TSV s 1% FA u prisutnosti 10% glicerola, pokusi dvostruke radijalne imunodifuzije pokazali su dva do četiri puta više virusnih čestica koje su sudjelovale u serološkoj reakciji negoli u preparatima obrađenim samo s 1% FA. Sukladno tome, metoda osigurava kvalitetnije preparate namijenjene za različite imunokemijske pokuse i biofizičke i biokemijske analize. Smatramo da će upotreba glicerola biti općenito korisna pri fiksiranju virusa koji pokazuju sklonost agregiranju u prisutnosti FA.

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