Properties of the Nigerian and Ivory Coast Strains of the Okra Mosaic Virus

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ABSTRACT

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Okra viruses from Ivory Coast and Nigeria have virtually identical biophysical properties and are closely related serologically. On the basis of spur formations in cross-serological tests, cross-serological reaction with other viruses of the tymovirus group, and differential host susceptibility, they are considered to be related, but distinct, strains of the same virus.

Additional key words: buoyant density, turnip yellow mosaic virus, cocoa yellow mosaic virus, *Clitoria* yellow vein virus, Kennedia yellow mottle virus, *Desmodium* yellow mottle virus, eggplant mosaic virus.

The viruses described as okra mosaic virus from Ivory Coast (IC-OkMV) (6) and Nigeria (N-OkMV) (10, 11) are similar in properties, but there is no previously published serological or biophysical comparison of the two isolates. Reports concerning IC-OkMV by Koenig (8) and Givord and Koenig (7) referred to a Nigerian strain without defining its origin. The former mentioned only electrophoretic mobility and the latter referred only to the host range of undefined Nigerian isolates. On the basis of these two publications, some virologists have prematurely concluded that N-OkMV (6) is the same virus as IC-OkMV (10, 11).

This note reports the results of an interlaboratory project to compare IC-OkMV and N-OkMV. It was found that the two are, in fact, closely related but distinct strains of the same virus. In addition the density in CsCl of the viral components and sedimentation coefficients (previously reported values were S_{obs} in contrast to $S_{20,w}$) are reported for the first time.

MATERIALS AND METHODS

Propagation of the viruses.—The Nigerian strain of okra mosaic virus (N-OkMV) (10) was maintained in cowpea (*Vigna unguiculata* 'Cal. No. 5') and purified by extraction with 0.1 M potassium phosphate buffer, pH 6.0, and *n*-butanol, followed by two cycles of differential centrifugation using 10 min at 10,000 *g* for low-speed and 2.5 hr at 74,000 *g* for high-speed centrifugation.

For antiserum production the virus was further purified by zonal centrifugation in a Beckman Ti-15 zonal rotor. A 10-40% approximately linear gradient was centrifuged at 30,000 rpm for 4 hr and fractionated by pumping through an ultraviolet monitor. Rabbit antiserum was prepared against purified bottom component and purified top component by a combination of intravenous injections and intramuscular injections using Freund's complete adjuvant (Difco Laboratories, Detroit, MI 48232).

The Ivory Coast strain (IC-OkMV) was purified by the same method from cucumber (*Cucumis sativus* 'National Pickling') for the biophysical studies. The purification for antiserum production has been described previously (9).

Analytical methods.—The method used for the preparation and analysis of sucrose density gradients has been described previously (10).

Analytical ultracentrifugation was done in a Beckman analytical ultracentrifuge equipped with an ultraviolet scanner and electronic speed control. Samples were dialyzed 2 days against three changes of 0.1 M pH 7.0 phosphate buffer and centrifuged at 20,000 rpm in an ANF-Ti rotor. Scans were made at 4-min intervals. Samples analyzed by ultraviolet optics were considered at infinite dilution. Procedures described by Chervenka (3) were used.

Viscosity of buffers was determined using a Cannon-Ubbelohde semimicro viscometer, and buffer densities were determined in a 50.0-ml pycnometer. The density in CsCL gradients was carried out as previously described in the analytical ultracentrifuge (3) and in the Beckman SW 50.1 preparative rotor (1).

For analysis of virus protein, virus suspensions at a concentration of about 2 mg/ml were incubated in 4 M urea and 2% SDS at 45 C for 45 min. Ten- to 20-μliter samples were electrophoresed 4 hr on 7% polyacrylamide gels at 5 mA per gel (4). The proteins of tobacco mosaic virus, turnip yellow mosaic virus, chymotrypsinogen, and ovalbumin were used as standards. Gels were washed with

TABLE 1. Properties of the centrifugal components (top and bottom) of okra mosaic virus from Nigeria (N-OkMV) and Ivory Coast (IC-OkMV)

Characteristics	N-OkMV		IC-OkMV	
	Top	Bottom	Top	Bottom
Diameter of virion (nm)	28.1 ± 1.0	28.1 ± 1.0	28.1 ± 1.0	28.1 ± 1.0
$S_{20,w}$	52.03 ± 3.71	112.42 ± 1.85	51.76 ± 0.75	113.21 ± 1.93
p-CsCl (prep. centrif.)	1.252	1.445	1.253	1.448
p-CsCl (anal. centrif.)	1.259	1.442	1.255	1.436
Protein subunit molecular weight	20,000	20,000	20,000	20,000

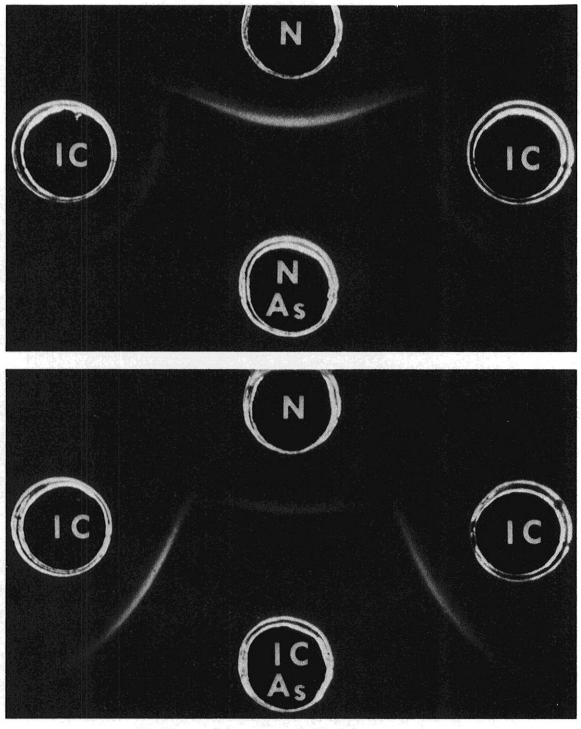

Fig. 1. Double diffusion serological tests between strains of okra mosaic virus from Ivory Coast (6) and Nigeria (10): IC and N are the respective antigens and IC-As and N-As are the respective antisera.

10% trichloroacetic acid (TCA), stained with 0.05% Coomassie blue in TCA, and destained with 10% TCA prior to being scanned at 560 nm on a 2400-S spectrophotometer (Gilford Instrument Laboratories, Oberlin, OH 44074) equipped with a linear transporter.

For electron microscopy, carbon-coated Formvar grids were floated on drops of virus suspension, rinsed twice with H_2O, and stained with 1% uranyl acetate. Electron micrographs were taken in a Zeiss EM 9-2S electron microscope calibrated by grid replica.

Serological analysis by Ouchterlony double diffusion and immunoelectrophoresis have been described previously (2, 8, 9). The preparation of antisera to cocoa yellow mosaic virus (CoYMV), turnip yellow mosaic virus (TYMV), *Clitoria* yellow vein virus (CYVV), Kennedia yellow mottle virus (KYMV), and *Desmodium* yellow mottle virus (DeYMV) have been described previously (8). Purified eggplant mosaic virus (13) and antiserum were supplied by H. Waterworth, of the U.S. Department of Agriculture, Plant Industry Station, Glen Dale, MD 20705.

RESULTS AND DISCUSSION

Table 1 summarizes the results of the comparative analysis of N-OkMV and IC-OkMV. The viruses were nearly identical in all properties analyzed and also were similar to turnip yellow mosaic virus (12), the type virus of the tymovirus group (5).

Serological tests carried out at Boyce Thompson Institute using antiserum to N-OkMV and IC-OkMV at a dilution of 1:50 resulted in a strong serological cross reaction. When N-OkMV and IC-OkMV were placed in adjacent antigen wells, a weak spur was produced with either antiserum (Fig. 1). Neither TYMV nor eggplant mosaic virus reacted with either antiserum. Neither N-OkMV nor IC-OkMV reacted with antiserum to eggplant mosaic virus.

In Braunschweig, extensive serological tests were made with a number of viruses and antisera of the tymovirus group. These analyses indicated that IC-OkMV and N-OkMV are closely related serologically (Table 2). An attempt was made to detect a serological difference between them by reaction with antisera to IC-OkMV from early and late bleedings. With antisera from rabbit number 388 there was a difference in titre to OkMV but not with rabbit number 387. The titres of these antisera from rabbit 388 were one twofold dilution step higher with the homologous than with the heterologous antigen. A faint spur was observed when the two antigens were tested side by side with these antisera.

Koenig and Givord (9) previously reported an improved differentiation of closely related tymoviruses with heterologous antisera. The test was applied to IC-OkMV and N-OkMV. A serological cross-reaction test was made between IC-OkMV and N-OkMV antigen and antisera to five other members of the tymovirus group (Table 2).

Twenty-seven bleedings from nine different rabbits immunized with either CYVV, KYMV, or DeYMV reacted the same to both okra virus antigens. On the other hand, sera of rabbits immunized with CoYMV or TYMV clearly differentiated between the two isolates (Table 2). In fact, the antiserum to TYMV did not react with N-OkMV but it did react with IC-OkMV. When the two

TABLE 2. Serum dilution endpoints of antisera to members of the tymovirus group which reacted with the Nigerian (N-OkMV) and Ivory Coast (IC-OkMV) isolates of okra mosaic virus

Antisera to:[a]	Serial no. of rabbit	Titre with antigen		
		IC-OkMV	N-OkMV	Homologous
IC-OkMV	388	2,048	1,024	2,048
IC-OkMV	387	2,048	2,048	2,048
CoYMV	440	4	16	2,048
CoYMV	442	1	8	256
TYMV	91	4	0	256
CYVV	As[b]	64	64	1,024
KYMV	As[b]	128	128	4,096
DeYMV	As[b]	16	16	512

[a]Isolate IC-OkMV, okra mosaic virus from Ivory Coast; CoYMV, cocoa yellow mosaic virus; TYMV, turnip yellow mosaic virus; CYVV, Clitoria yellow vein virus; KYMV, Kennedia yellow mottle virus; DeYMV, Desmodium yellow mottle virus.

[b]Similar values with nine bleedings from three different rabbits each.

isolates were tested side by side with CoYMV antisera, spurs were observed. Antiserum from a third rabbit also immunized with CoYMV did not differentiate the two OkMV isolates as well as those listed in Table 2.

A distinct difference in host range and symptomatology was observed at Boyce Thompson Institute. A mosaic was produced by IC-OkMV in cucumber cultivar National Pickling, but it did not infect cowpea cultivar Cal. No. 5. Givord and Hirth (6) reported that only 30 of 60 cowpea plants inoculated with IC-OkMV showed symptoms. On the other hand, N-OkMV produced severe mosaic symptoms in cowpea and was symptomless in cucumber. In Braunschweig, both N-OkMV and IC-OkMV produced a severe mosaic in cucumber cultivar Delikatess. Negative results in infectivity tests were confirmed by serology and by recovery tests.

This study should leave no doubt as to the relationship of these two virus strains. The physical properties of the two were identical within the ability of the techniques used to show differences. Mixtures of viruses co-migrated toward the cathode at pH 7.8 in immunoelectrophoresis experiments at Boyce Thompson and at pH 7.0 at Braunschweig. The sedimentation coefficients reported in Table 1 were measured at essentially infinite dilution and therefore supersede previously reported values (6, 10). The serological tests showed that isolates are closely related but distinct.

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