Assessment of Southern Bean Mosaic Virus Monoclonal Antibodies for Affinity Chromatography

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ABSTRACT

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The dissociation of immunoprecipitates of three monoclonal antibodies (B5, B6, and B10) with southern bean mosaic virus was assessed at pH values between 7.0 and 2.25 by density gradient analysis and by indirect ELISA. Both methods showed that B10 did not dissociate appreciably, B5 dissociated at pH 2.75–2.25, and B6 dissociated at pH 3.5–2.25. Purified virus was recovered from a B6 affinity column by pH gradient elution at pH 3.4–2.2 or by elution of virus swollen with 2 mM EDTA in pH 7 buffered saline. Virus partially purified by polyethylene glycol or by chromatography on Sephacryl S-300 was recovered by pH gradient elution, but clarified sap of infected plants destroyed the reactivity of the B6 affinity column. Electron microscopy showed virus particles on B6 agarose beads at higher pH values and the release of virus into lower pH washes.

A panel of monoclonal antibodies against the bean-type strain (SBMVb) and the cowpea-type strain (SBMVc) of southern bean mosaic virus has been assessed for reactivity with bean and cowpea strains and with the virus antigen in different conformations (5,6,8). Only three of the SBMVb monoclonal antibodies, B5, B6, and B10, precipitated the SBMVb virion. B5 is an IgM type and B6 and B10 are IgG types. In this study, the utility of these antibodies for virus purification by affinity chromatography was assessed. This study may be applicable to affinity chromatography of viruses that are in low concentration in the plant or are difficult to purify. A preliminary report of some results has been made (7).

MATERIALS AND METHODS

Dissociation of antigen-antibody precipitates. The sources of cultures of SBMVb and SBMVc, the virus purification method, and the preparation, purification, and properties of SBMVb monoclonal antibodies B5, B6, and B10 have been described previously (5,8). Virus-antibody precipitates were prepared by the addition of 300 μl of ascitic fluid in 3 ml of 0.85% NaCl (PS) to 1.05 mg of virus in 3 ml of PS. The mixture was kept at 4 C overnight, suspended, and 570-μl aliquots were dispensed into 10 Eppendorf tubes. Each precipitate was pelleted (2 min at 12,000 rpm in a Silencer H25 centrifuge), resuspended, and washed twice with 1 ml of PS containing 0.02% sodium azide. Pellets were resuspended overnight in 100 μl of 0.2 M glycine-HCl buffer at pH 2.25, 2.50, 2.75, 3.00, or 4.00. These samples were placed on 5–35% sucrose gradients in the same buffer and centrifuged at 38,000 rpm (246,000 *g*) in a Beckman SW 41 rotor for 1 hr at 4 C. The extent of dissociation of virus-antibody precipitates was monitored by the amount of virus observed in the gradients with an ISCO model 183 gradient fractionator and an ISCO model UA-5 absorbance monitor. These experiments were repeated with similar results.

Affinity column. Five milligrams of B6 antibody, purified by chromatography on Sephacryl S-300, was reacted with 1 ml of washed, swollen, CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) (1). The gel was packed in a 1 × 1 cm column, washed with 0.2 M sodium acetate, 0.85% NaCl, and 0.01% sodium azide, pH 7.0 (N7). Purified virus, 1–10 mg, in 250 μl of 0.01 M sodium phosphate buffer (pH 7.5) containing 0.15 M NaCl (PBS), was applied to the column and recirculated by pumping at 16.4 ml/hr for 30 min. The column was washed with N7 buffer, then a gradient of 50 ml of N7 and 50 ml of 0.2 M glycine-HCl, 0.85% NaCl, and 0.01% sodium azide, pH 2, (N2) was applied at 6.8 ml/hr.

For crude virus preparations, infected leaves were ground in 0.1 M sodium acetate buffer, pH 5.0, containing 5 mM DIECA (1 g of leaves per 4 ml of buffer) and squeezed through cheesecloth; after 18 hr the expressed sap was clarified by low-speed centrifugation. The pH was adjusted to 7.0 with NaOH, and the sample was recentrifuged and filtered through a 0.2-μm Metricel Gelman filter. Forty milliliters was recirculated through the column at 6.8 ml/hr for 14 hr. A similar preparation was passed through a Sephacryl S-300 column (2.5 × 75 cm) in PBS, and the voided material was applied to the column. Also, virus was precipitated from pH 5.0 clarified sap by 8% PEG 6000, dissolved in PBS, and then applied to the column. With the exception of the test with crude sap, all affinity chromatography experiments were repeated with similar results.

Electron microscopy. A 100-μl suspension of the B6 affinity column was mixed with 1 mg of SBMVb in PBS and centrifuged for 20 sec at 4,000 rpm in a Silencer centrifuge. The supernatant fluid was removed with a pipette, 100 μl of PBS was added, and the gel was gently resuspended with a vortex mixer. The washing was repeated three times with N7 and then with N7–N2 buffer mixtures at pH 6, 5, 4, 3, and 2. The supernatant fluids were placed on grids, stained with 2% uranyl acetate, and examined in a Hitachi H 600 electron microscope. After each wash, an aliquot of the B6 agarose beads was suspended in an equal volume of 2% uranyl acetate, placed on a grid, drained, and gently washed with 2% uranyl acetate. The beads were located in the electron microscope under low magnification; the beam was focused on an adjacent grid area, then on the bead. Frequently, the agarose beads disappeared during assessment of the number of attached virus particles and before pictures could be taken.

Serological methods and polyacrylamide gel electrophoresis. The methods for gel diffusion, indirect enzyme-linked immunosorbent assay (ELISA), and SDS polyacrylamide gel electrophoresis (SDS-PAGE) were described previously (5,8). The dissociation of monoclonal antibodies at various pH values from virus adsorbed to plates was monitored as follows: SBMVb was placed on plates and monoclonal antibody in six fourfold dilutions was added; the plates were washed twice for 30 min with acetate-glycine buffers (containing 0.2% bovine serum albumin and 0.05%

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Tween 20) at various pH values; the buffer was removed and enzyme-labeled goat antimouse antibody was added, then removed; substrate was added and absorbance was read in a Titertek Multiskan MC plate reader. The data were fitted by computer to an equation for ELISA reactions (8). The ELISA titer index was defined as the maximum absorbance attained (A_{max}) divided by the antibody dilution that produced one-half A_{max}. The results of ELISA experiments were reproduced in either identical or similar experiments done with changes in washing time or procedure.

Preparation of F(ab')$_2$ from B6. Five milligrams of lyophilized DEAE-Sephacel-purified B6 was dissolved in 2.25 ml of 0.1 M sodium citrate, pH 3.5, containing 0.002% sodium azide, and 250 μl of pepsin (250 μg/ml water) was added (2). After reaction for 18 hr at room temperature, the solution was chromatographed on a Sephadex G-100 column (2.5 × 38 cm) in PBS. Fractions containing the IgG and F(ab')$_2$ peaks were tested by indirect ELISA with SBMVb and by gel diffusion tests with SBMVb and SBMVc. The identity of the IgG and F(ab')$_2$ peaks was confirmed by SDS-PAGE (2).

RESULTS

Dissociation of virus-antibody precipitates. Based on the amount of virus released, SBMVb-B5 precipitates dissociated almost completely at pH 2.25 and 2.50 (Fig. 1). Much less virus was released at pH 2.75 and essentially none at pH 3.00. Very little dissociation of SBMVb-B10 precipitates occurred even at pH 2.25 and 2.50 (Fig. 1). SBMVb-B6 precipitates dissociated completely at pH 2.25 and 2.50, but progressively less virus was freed at pH 2.75 and 3.00 and none at pH 4.00 (Fig. 2). SBMVc-B6 precipitates differed from all others; they dissolved immediately on addition of the pH 2.25, 2.50, 2.75, and 3.00 buffers. Almost equal quantities of virus were observed in the gradients at all pH values (Fig. 2). Most of the released SBMVb sedimented more slowly at pH 2.25, as did SBMVc at pH 2.25 and 2.50 (Figs. 1 and 2). Similar sedimentation patterns were obtained with control virus preparations at these pH values (*unpublished*), and the slower sedimentation rate indicates virion swelling.

A fraction from the top of each gradient in these tests was adjusted to pH 7 with 1 M Tris and used in gel diffusion serological tests. SBMVb-reactive antibody was recovered from SBMVb-B5, SBMVb-B6, and SBMVc-B6 precipitates at pH 2.25 and 2.50 but not at pH 2.75, 3.00, or 4.00. SBMVc-reactive antibody was not recovered from SBMVb-B6 or SBMVc-B6 precipitates at any pH.

Reactivity of B6 with SBMVc. The unusual precipitation of SBMVc with B6 in gel diffusion tests (8) prompted us to investigate the reactivity of F(ab')$_2$ from B6 with SBMVc and SBMVb. The IgG fraction ($A_{280nm} = 0.052$) reacted with SBMVb and SBMVc in gel diffusion tests but the F(ab')$_2$ fraction ($A_{280nm} = 0.042$) reacted only with SBMVb. These results indicate that SBMVc complexes with the Fc region of B6 rather than with the antigen-combining site of B6.

Dissociation of antibody from virus on ELISA plates. The amount of monoclonal antibody remaining bound to virus coated on an ELISA plate after washing at pH 7, 6, 5, 4, 3.5, 3, 2.5, and 2.25 was measured (Table 1) as two parameters, percentage of the A_{max} at pH 7 and percentage of the ELISA titer index at pH 7. The percentage A_{max} shows dissociation of B5 at pH 3 and lower, dissociation of B6 at pH 3.5 and lower, and no appreciable dissociation of B10. These results agreed with the precipitate dissociation data in Figures 1 and 2.

The percentage ELISA titer index decreased to 50% for B10 at pH 3.5 and lower, indicating some removal of B10 from the plate.

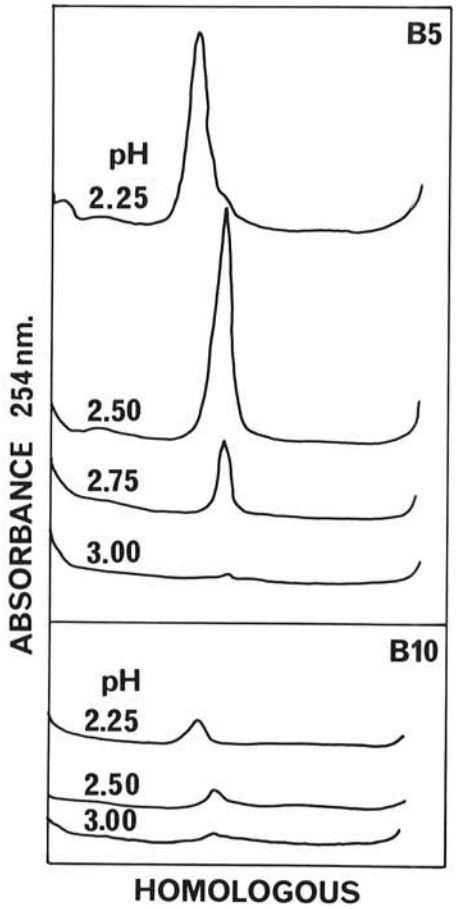

Fig. 1. Effect of pH on the dissociation of SBMVb-B5 and SBMVb-B10 (homologous) immunoprecipitates (SBMVb = southern bean mosaic virus, bean-type strain). Precipitates were placed on the gradients at the pH indicated and gradients were centrifuged at 4 C for 1 hr in an SW 41 rotor, then scanned at 254 nm. Sedimentation is from left to right.

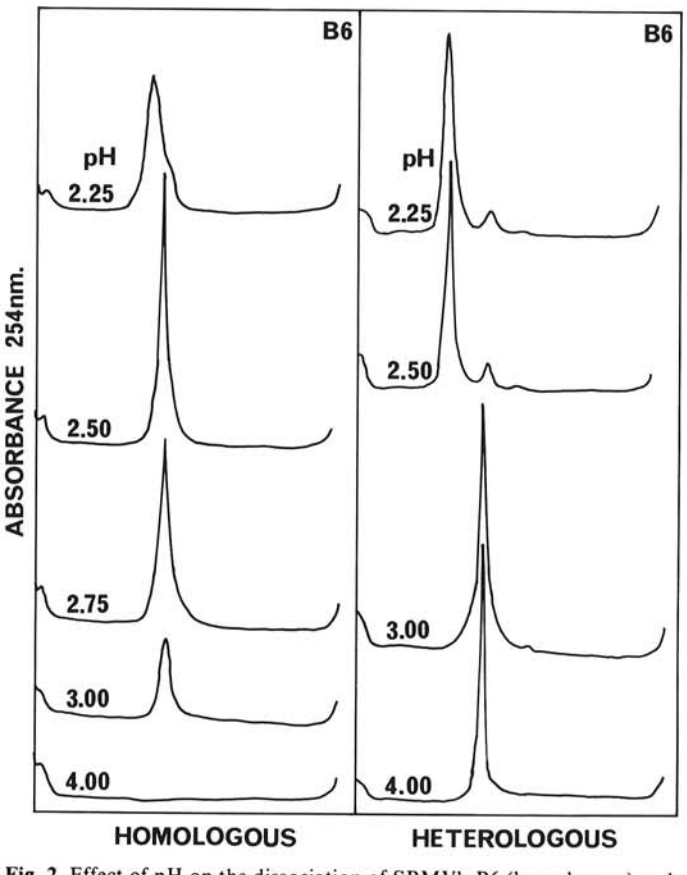

Fig. 2. Effect of pH on the dissociation of SBMVb-B6 (homologous) and SBMVc-B6 (heterologous) immunoprecipitates (SBMVb = southern bean mosaic virus, bean-type strain; SBMVc = southern bean mosaic virus, cowpea-type strain). Precipitates were placed on gradients at the pH indicated and gradients were centrifuged at 4 C for 1 hr in an SW 41 rotor, then scanned at 254 nm. Sedimentation is from left to right.

Larger decreases were observed with B5 at pH 2.5 and 2.25 and with B6 at pH 3.5, 3.0, 2.5, and 2.25. These results demonstrated that the ELISA titer index was more sensitive to removal of antibody or virus-antibody complex than was A_{max}.

Affinity chromatography. One milligram of purified SBMVb was applied to the B6 affinity column, and a sharp virus peak was eluted at pH 3.8–2.2 in the gradient (Fig. 3). To determine the capacity of the column, 10 mg of SBMVb was applied, excess virus was eluted with N7, and 2.5 mg of virus was eluted by the gradient at pH 4.3–2.2. The B6 affinity column bound similar quantities of SBMVb applied at pH 5.0.

Antigen inhibition studies (5) demonstrated B6 did not react with swollen SBMVb in solution, but B6 did react with swollen SBMVb on polystyrene plates in indirect ELISA (8). One milligram of purified SBMVb was applied to the B6 affinity column, and after a wash with N7, N7 containing 2 mM EDTA was used as an eluant (Fig. 4). The virus was eluted in a single peak that was somewhat broader than the peak in Figure 3.

Purified SBMVc was not retained on the B6 affinity column (*unpublished*). When 1 mg of SBMVc was applied, the virus was eluted in N7 and appeared denatured or aggregated. This virus reacted with polyclonal SBMVc antiserum, and native virus particles were observed with the electron microscope.

SBMVb partially purified by PEG precipitation or by chromatography on Sephacryl S-300 was applied to the column and eluted in the pH gradient. The elution patterns obtained were similar to that shown in Figure 3, but much more UV-absorbing material was eluted in the N7 wash. Based on subsequent trials with purified SBMVb, the capacity of the B6 affinity column was unaltered by these runs. Application of clarified sap from SBMVb-infected plants over a 14-hr period destroyed 85% of the virus-binding capacity of the affinity column. The loss of activity was probably caused by proteases or polyphenols in the plant sap. The extended period of contact of the column with plant sap would be essential in purification of viruses in low concentration.

Electron microscopy of affinity gels. Many virus particles were found in electron micrographs of the first two N7 washes of the SBMVb-reacted B6 agarose beads. Few particles were observed at pH 6.0 and 5.0. Increasing numbers of particles were found at pH 4.0 and 3.0, and a large number of particles were found at pH 2.0.

Examination of the B6 agarose beads after the pH 5.0 wash showed virus particles attached to agarose beads (Fig. 5). The agarose particles often broke during preparation, and many small agarose particles were observed. The group of eight virus particles at the bottom of Figure 5 is probably attached to agarose peeled from a bead.

DISCUSSION

Precipitate dissociation on density gradients (Fig. 1) and estimation of virus-antibody dissociation by ELISA (Table 1) demonstrated that B5 and B10 would not be useful for affinity chromatography purification of native virus by low-pH elution. However, SBMVb-B6 precipitates were dissociated at higher pH values that did not denature the virion significantly. Since the titers of purified B10, B5, and B6 (the reciprocal dilution that yielded one-half the maximum absorbance at 405 nm in ELISA) were 1,800, 1,600, and 20,000, respectively (8), antibody titer does not correlate with the pH of dissociation. The indirect ELISA method could be used in screening monoclonal antibody culture fluids to obtain antibodies for affinity chromatography. This method may also be applicable to screening for dissociation of virus-antibody complexes with chaotropic ions, urea, guanidine, or polarity-reducing agents (1).

The reactivity of B6 with SBMVc in gel diffusion tests contrasts to its weak reactivity with SBMVc in indirect ELISA and its lack of

TABLE 1. The effect of pH of wash on the removal of monoclonal antibody bound to southern bean mosaic virus, bean-type strain, coated on an ELISA plate

	Percentage of A_{max} at pH 7			Percentage of ELISA titer index[a] at pH 7		
Wash pH	B5	B6	B10	B5	B6	B10
7.0	100	100	100	100	100	100
6.0	96	95	97	60	93	86
5.0	93	96	95	67	75	85
4.0	88	87	89	70	50	75
3.5	98	76	88	49	30	56
3.0	77	46	80	54	5	54
2.5	33	30	97	22	2	58
2.25	24	29	82	20	3	56

[a]The maximum absorbance attained (A_{max}) divided by the antibody dilution that produced one-half A_{max} (8).

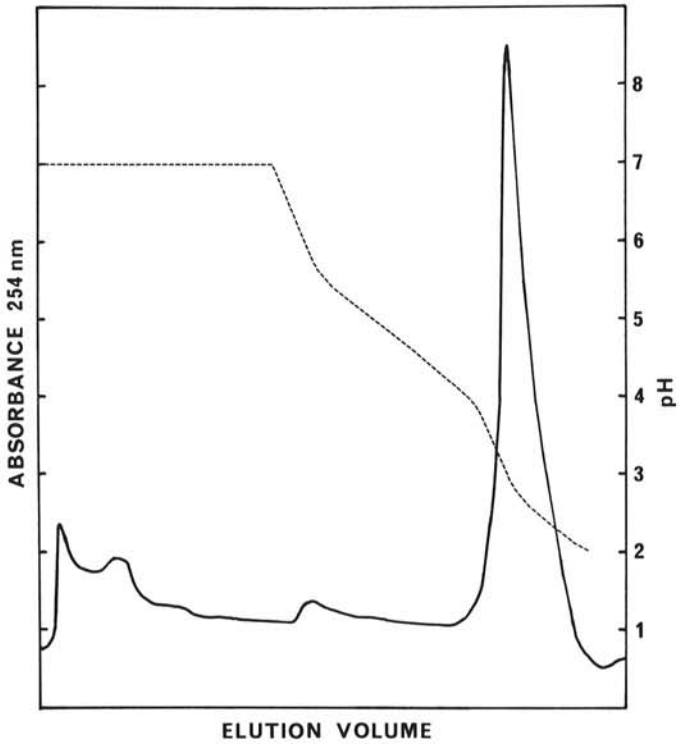

Fig. 3. Elution of southern bean mosaic virus, bean-type strain, from a B6 affinity column (1 × 1 cm) with a pH gradient; —— absorbance at 254 nm, ····· pH of eluant.

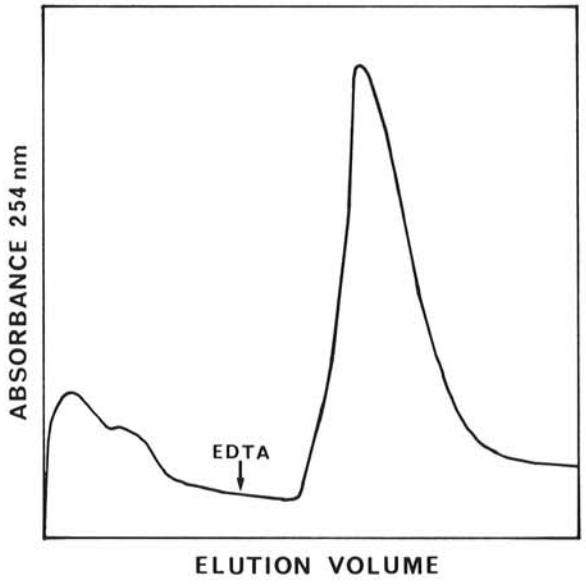

Fig. 4. Elution of southern bean mosaic virus, bean-type strain, from a B6 affinity column (1 × 1 cm) with pH 7 buffer containing 2 mM EDTA. Arrow indicates start of EDTA buffer.

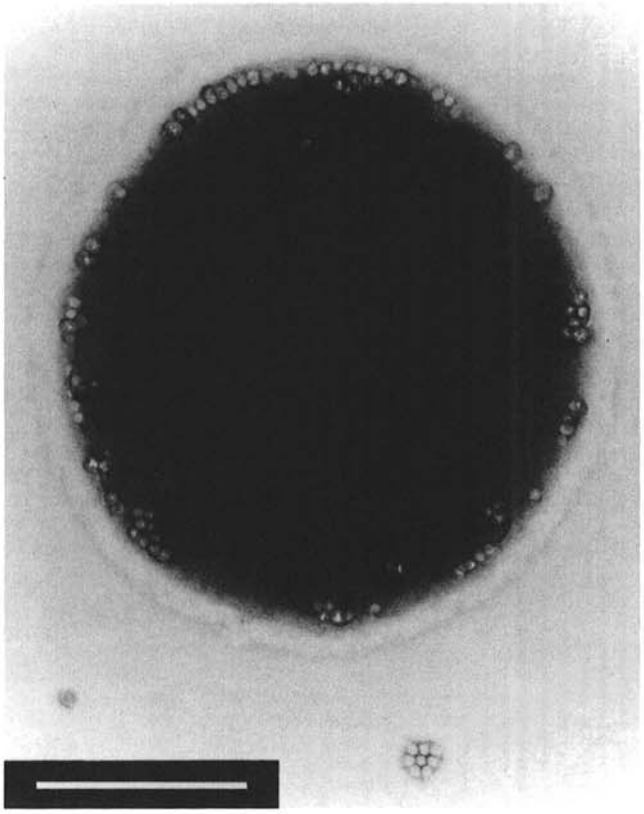

Fig. 5. Electron micrograph of an agarose bead from a B6 affinity column showing attached particles of southern bean mosaic virus, bean-type strain. Bar = 500 nm.

reactivity in antigen inhibition ELISA (5,8). Loss of reactivity of B6 after conversion to $F(ab')_2$ indicates that the SBMVc may bind to the Fc region of B6. The interaction between SBMVc and B6 was sufficient to maintain the SBMVc-B6 precipitates after several washes in PS because SBMVb-reactive antibody was recovered from the top of density gradient columns in Figure 2.

The B6 affinity chromatography with partially purified SBMVb was successful. However, SBMVb is much more stable in acidic solutions (3) than, for example, barley yellow dwarf virus, a prime candidate for purification by affinity chromatography (4). Affinity chromatography with some viruses that are in low yield or difficult to purify may not be applicable to the recovery of native virions. However, it may provide sufficient denatured virus for use as source of nucleic acid for recombinant DNA research.

The swelling of SBMVb in EDTA allowed its recovery from the B6 affinity column at pH 7. Sobemoviruses, tombusviruses, bromoviruses, dianthoviruses, and tobacco necrosis satellite virus swell, but structural properties of many other viruses (e.g., cryptic viruses) have not been determined. Indeed, it may be possible to dissociate some virus-monoclonal antibody interactions by relatively minor alterations in virus structure.

Electron microscopy rapidly assessed the release of SBMVb from B6 agarose beads, but indirect ELISA was more quantitative. The examination of B6 agarose beads for attached virus by electron microscopy was hampered by the fragile nature of the beads in solution and in the electron microscope beam. Affinity beads are feasible for detecting low levels of virus but probably do not offer specific advantages over current IEM procedures.

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