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CHARACTERISATION OF RUBELLA VIRUS HEMAGGLUTININ ROSETTES

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(Accepted 22 August 1980)

Purified rubella virus treated with Triton X-100 was subjected to centrifugation in a sucrose density gradient containing nonionic detergent β -D-octylglucoside. The result of this treatment was the formation of hemagglutinating rosettes containing viral glycoproteins VP₂ (50,000 mol. wt.) and VP₃ (63,000 mol. wt.). The rosettes have a 26 S sedimentation coefficient and a density of 1.2 g/cm³ in sucrose. Electron microscopy revealed 15 nm rosettes with a hollow center. The molecular weight of the rosettes was extrapolated at 850,000.

INTRODUCTION

Rubella virus prophylaxis leads to attempts to develop a subunit vaccine because of the fetal consequences of maternal rubella immunisation with live virus (Fleet et al., 1974). In these attempts, partially purified rubella virus is disrupted by treatment with nonionic detergents Tween 80 or Nonidet P-40. The hemagglutinin composed of two glycoproteins, VP₃ and VP₂, is then separated on sucrose density gradient (Schmidt et al., 1968; Väänänen and Vaheri, 1971; Cappel and De Cuyper, 1976). In these reports, the physical and biological state of the partially purified hemagglutinin is poorly characterised, thus making it impossible to correlate the efficiency of these vaccines. Furthermore, immunising doses were either very high or in multiple injections.

Morein et al. (1978) shows that, depending on the physical structure, lethal infection of mice can be prevented by a single injection with virus spike protein (10 μ g) if injected in rosette form. It is thus very important to characterise the physical state of the viral subunits prepared for vaccination. Many published papers describe rosette formation by isolated membrane glycoproteins of enveloped viruses (Laver and Valentine, 1969; Shimuzu et al., 1972; Kitano et al., 1974; Brady and Furminger, 1976; Morein et al., 1978; Schneider et al., 1979; Collins and Alexander, 1980; Sturman et al., 1980).

The present report describes the method of preparation, the morphology, composition, sedimentation coefficient and buoyant density of rubella virus hemagglutinin rosettes.

MATERIAL AND METHODS

Virus production and concentration

Rubella virus, strain M-33 (ATCC VR-315) was grown in Vero cells (a continuous heteroploid line of African green monkey kidney cells) produced in a multiple tube tissue culture propagator (TM-15: Bellco Glass Co., Vineland, N.J.) (Corbeil et al., 1979). Confluent cell cultures were infected at a multiplicity of one TCID₅₀ per cell. Supernatants were harvested every two days, and those containing 8-32 HAU (hemagglutinating units) 2.5 µl were pooled and the viral particles were concentrated using two successive cycles of hollow fiber ultrafiltration on Amicon model DC-10 and DH-4 systems (Amicon, MA) (Trudel and Payment, 1980). The ultrafiltration system was modified with quick-connect fittings and air filters to prevent aerosol dispersion. The concentrator was equipped with H1-100 hollow fiber cartridge (molecular weight cut-off: 100,000).

After ultrafiltration the residual volume was 50 ml. The concentrated viral suspension was brought to 10 mM EDTA (using a 1.0 M solution) to disaggregate the virions. The larger particles were removed by centrifugation at 10,000 g for 15 min. Further concentration was achieved by hydroextraction with polyethylene glycol 20,000 (Carbowax 20,000). The final volume in the dialysis tubing was 2 ml. Virions were purified by gel filtration on Sepharose 2B 2.5 × 40 cm column; NTE buffer (0.15 M NaCl; 0.05 M Tris-HCl; 0.001 M EDTA; pH 7.4) was used as the eluent at a flow rate of 10 ml/h. Fractions of 3 ml were collected and assayed for hemagglutinating activity and optical density at 280 nm.

Hemagglutinating assay

Rubella hemagglutination assays were performed in Cooke Engineering microtiter round bottom disposable plastic plates as described previously (Trudel et al., 1979). The plates were incubated at 4°C for 1 h. One unit of antigen was defined as the highest dilution that produced complete hemagglutination of one day old chick erythrocytes.

Disruption of virus by nonionic detergents

Chromatography-purified virus was disrupted by treatment with nonionic detergent Triton X-100 1% for 20 min which was then exchanged for the more easily dialysable β-D-octylglucoside (Calbiochem, California) (Morein et al., 1978; Helenius and Kartenbeck, 1980) by centrifugation through a 10–40% sucrose gradient containing 30 mM octylglucoside (SW40 rotor, 4 h at 40,000 r.p.m.). Fractions (0.3 ml) were collected and tested for hemagglutinating activity.

Purification of rosettes

To further purify the disrupted hemagglutinin and exchange the sucrose and detergent for NTE buffer, fractions showing hemagglutinating activity were pooled and 1 ml purified by chromatography on a Sepharose 6B column (0.5 × 15 cm).

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis in reducing conditions was performed according to the method of Weber et al. (1972). Gels were stained either with Coomassie blue R250 or PAS (periodic acid–Schiff) according to the method of Fairbanks et al. (1971).

Electron microscopy

Fractions, positive for hemagglutinating activity, were examined after negative staining with phosphotungstic acid 3%, pH 6, with a Phillips EM 300 microscope.

Rate zonal ultracentrifugation

The hemagglutinins were analysed by rate zonal centrifugation through a 10–40% sucrose gradient using ribosomal RNA 6S and 23S markers (SW41 rotor, 2 h, 40,000 r.p.m.). Buoyant density was determined by isopycnic banding in a 10–50% sucrose gradient (SW41 rotor, 18 h, 40,000 r.p.m.). Sucrose density was determined by refractometry.

RESULTS

As shown in Fig. 1, concentrated rubella virus chromatographed on Sepharose 2B eluted in 8 fractions of 2 ml (fractions 20–28) that corresponded to a very small protein peak. Rubella virions were structurally intact and recovery of virus-associated hemagglutinin was 70%.

Results of the solubilisation of rubella virus with nonionic detergents Triton X-100 and β -D-octylglucoside are summarised in Table 1. Octylglucoside alone at 30 mM or 100 mM was not efficient in disrupting the virus. Hemagglutinating activity could still be pelleted under conditions that permitted only sedimentation of whole virions. Treatment with Triton X-100 was also inefficient unless centrifugation through sucrose was performed. Combination treatment with 1% Triton X-100 for 20 min and then an exchange centrifugation through a 10–40% sucrose gradient containing 30 mM octylglucoside permitted efficient recovery of hemagglutinin associated in rosettes.

Sucrose-isolated rosettes chromatographed on Sepharose 6B eluted in a single well defined peak (Fig. 2) free of detergent and sucrose. Electron microscopic examination of peak material revealed rosette structures of 15 nm with a hollow center (Fig. 3). The

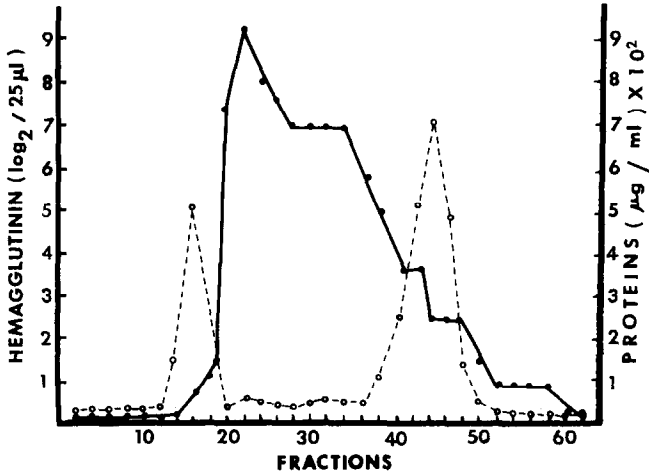


Fig. 1. Chromatography of rubella virus on a Sepharose 2B, 2.5×40 cm column: eluent, NTE buffer, pH 7.4; flow rate, 10 ml/h; fraction size, 3 ml.

TABLE 1

Solubilization of rubella virus with nonionic detergents Triton X-100 and β -D-octylglucoside
 Virus (1 ml, 2^8 HAU/0.025 ml)

Treatment	Conc.		Volume (ml)	Titer (HAU)	Recovery (%)
1. β -D-octylglucoside	30 mM	supernatant	1	2^5	12.5
		pellet	1	2^9	200
	100 mM	supernatant	1	2^7	50
		pellet	1	2^9	200
Triton X-100	1%	supernatant	1	2^7	50
		pellet	1	2^9	200
2. Triton X-100 + β -D-octylglucoside in sucrose gradient	1%	positive	1	2^5	
	30 mM	fractions	3	2^6	162
				2	

number of subunits in a rosette could not be determined accurately because of the small size of the hemagglutinin (5–6 nm). Polyacrylamide gel electrophoresis confirmed the presence of viral glycoproteins VP₃ (63,000 mol. wt.) and VP₂ (50,000 mol. wt.) associated with the rosettes.

Isopycnic banding in sucrose showed that the rosettes banded at 1.2 g/cm^3 . Rate zonal centrifugation analysis revealed that the rosettes migrated at the 26S position. Based on a 26S sedimentation coefficient the predicted molecular weight of the rosettes should be about 850,000.

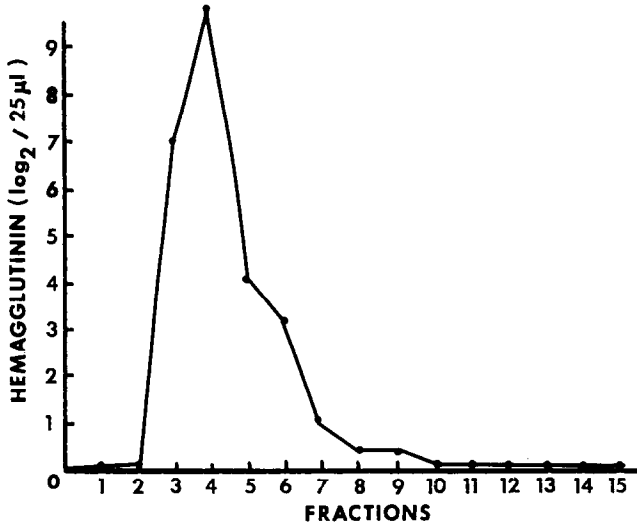


Fig. 2. Chromatography of rubella rosettes (hemagglutinin) on a Sepharose 6B column: eluent, NTE buffer, pH 7.4; flow rate, 2 ml/h; fraction size, 1 ml.

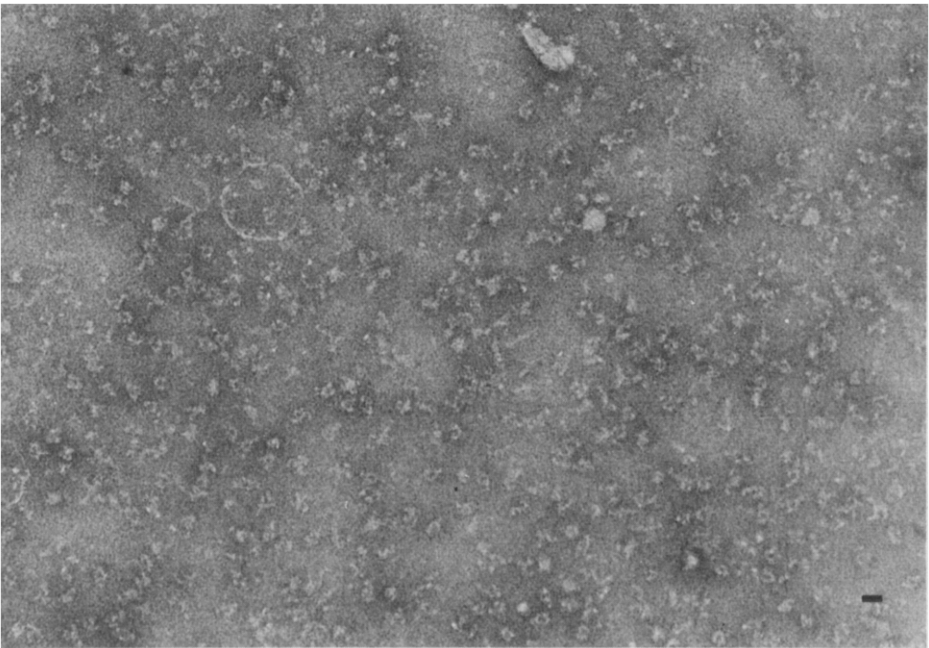


Fig. 3. Electron micrograph of rubella hemagglutinin rosettes purified by sucrose gradient followed by chromatography on Sepharose 6B. Scale bar is 25 nm.

DISCUSSION

This paper reports the isolation and characterisation of hemagglutinating rosette structures after detergent solubilisation of the envelope of rubella virus. These rosettes contain viral glycoproteins VP₃ and VP₂.

Rosette particles are also isolated by detergent extraction from coronavirus (Sturman et al., 1980), Friend leukemia virus (Schneider et al., 1979), Semliki Forest virus (Helenius and Von Bonsdorff, 1976), Japanese encephalitis virus (Kitano et al., 1974), avian myeloblastosis virus (Bolognesi et al., 1972), Sendai virus (Shimuzu et al., 1972), and influenza virus (Laver and Valentine, 1969). These particles have striking similarities to rubella virus rosettes in structure, buoyant densities and sedimentation coefficients.

The estimated molecular weight of the rosettes was 850,000. Taking into account the molecular weight of the rubella hemagglutinin glycoproteins (VP₃ (63,000 mol. wt.) + VP₂ (50,000 mol. wt.) = 113,000 mol. wt.) which are reported to be in equal amounts (Payment et al., 1975), the rosettes are estimated to be formed by eight subunits. This hypothesis will have to be investigated.

We have also found that effective solubilisation of rubella virus hemagglutinin and efficient removal of detergent needed an initial solubilisation with Triton X-100 and replacement with octylglucoside as described by Morein et al. (1978) and Helenius and Kartenbeck (1980) for Semliki forest virus. Octylglucoside unlike Triton X-100 is completely removed by dialysis.

Rosettes have been reported better immunogens than monomeric proteins (Morein et al., 1978). We are now evaluating these structures in order to prepare an efficient rubella subunit vaccine.

ACKNOWLEDGMENTS

This work was jointly supported by a grant from the National Research Council of Canada (Rubella 601) and the Medical Research Council of Canada (MA 6682).

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