Serological relationship of rice tungro spherical virus and bacilliform virus components associated with rice tungro disease

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Immunosorbent electron microscopy revealed a degree of serological relationship between rice tungro spherical virus and bacilliform virus indicating the existence of some common epitopes on the capsid protein of the two particles.

ETIOLOGICAL studies on rice tungro disease led to the discovery of association of two morphologically and serologically distinct rice tungro spherical virus (RTSV) and bacilliform virus (RTBV)[1,2]. However, a recent publication[3] pointed out certain similarities in the biological and intrinsic structural properties of these two viruses. It was, therefore, considered worthwhile to reexamine serological relationship of these particles.

RTSV particles with and without RTBV particles respectively were purified following Mishra *et al.*[4] and the modified Saito's procedure[5] standardized in this laboratory (Niazi *et al.* unpublished). For preparing the antisera separately of RTSV with and without RTBV, rabbits were immunized separately with purified preparations by three intravenous injections followed by two intramuscular injections at weekly intervals. For intramuscular injections, antigen was mixed with equal volume of Freund's incomplete adjuvant. Rabbits were bled one week after the last injection for obtaining the antisera.

To study the serological relationship, combined immunosorbent electron microscopy (ISEM) and decoration technique[6] was followed and various combinations of coating, trapping and decoration were tested (Table 1).

The present studies indicate that S+B antibodies trapped both S+B antigens in the ratio of 5:3 from clarified diseased sap, while S antibodies not only trapped S antigens but also B antigens from clarified diseased sap and purified preparations of S+B antigens in the ratio of 7:1 (Figure 1*a*). In further experimenta-

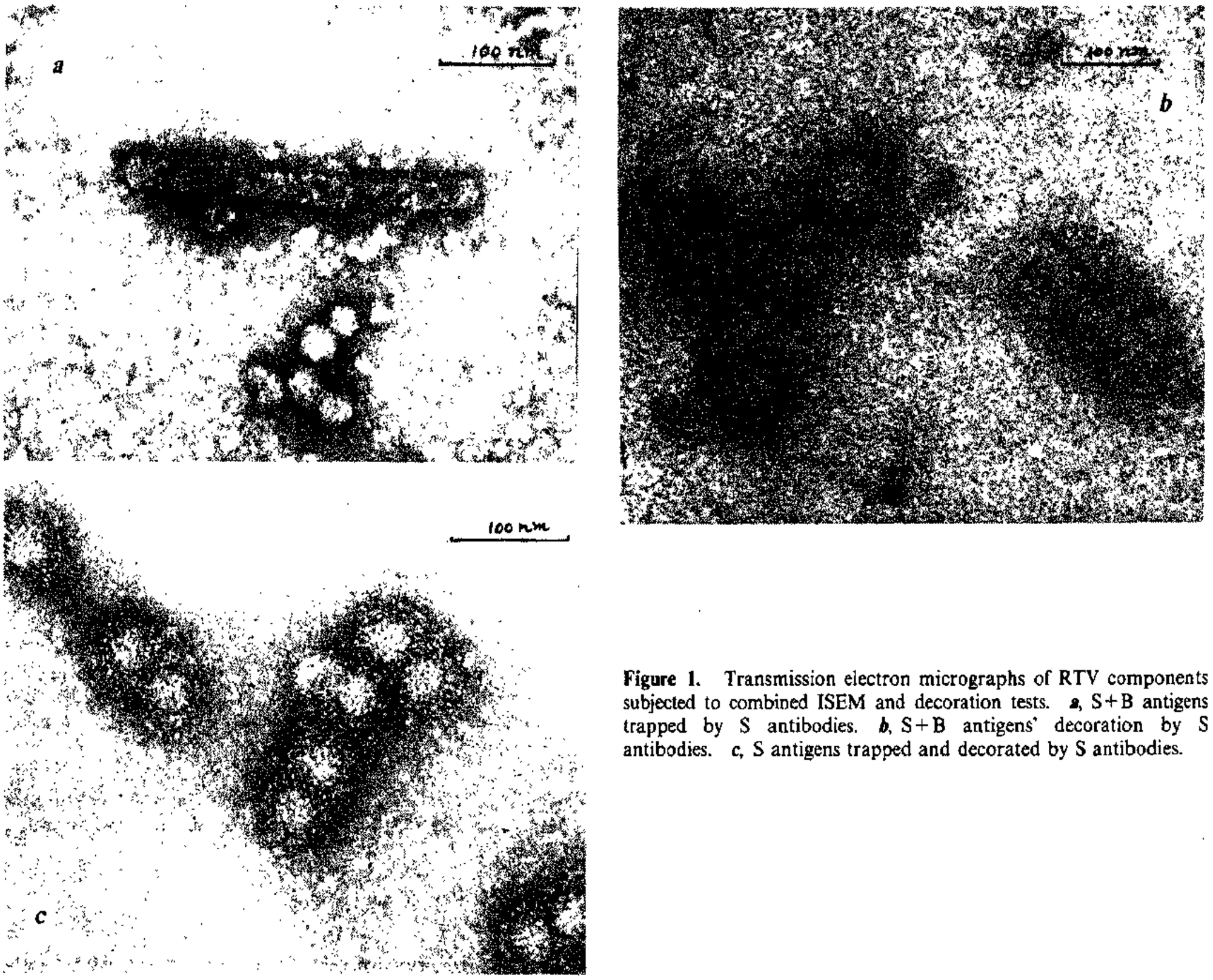

Figure 1. Transmission electron micrographs of RTV components subjected to combined ISEM and decoration tests. *a*, S+B antigens trapped by S antibodies. *b*, S+B antigens' decoration by S antibodies. *c*, S antigens trapped and decorated by S antibodies.

Table 1. Antigen–antibody combinations used for combined ISEM and decoration tests.

Combi-nations	Coating (1/320)	Trapping	Decoration (1/4)	Electron microscopic observations
1.	S antibodies	S antigens (purified)	S antibodies	Trapping and decoration of S antigens (Figure 1*c*)
2.	S antibodies	S+B antigens (purified and clarified diseased sap) (Figure 1*a*)	S+B antibodies	Trapping and decoration of S+B antigens
3.	S+B antibodies	S+B antigens (clarified diseased sap)	S antibodies	Trapping and decoration of S+B antigens (Figure 1*b*)

Antisera titre: S = 1:1024, S+B = 1:1024 (based on tube precipitin test).

tions of decorating the trapped antigens, S antibodies also decorated B antigens besides S antigens (Figure 1*b*). A point of interest here is the trapping and decoration of B antigens by S antibodies (Table 1), indicating some degree of positive serological relationship between the two morphologically distinct RTV components.

Earlier observations of Omura *et al.*[1] that the components of RTV are antigenically distinguishable were based on ouchterlony and clumping experiments. However, the present findings indicate antigenic relationship between the two particles at particulate level, probably due to the existence of some common epitopes in the capsid protein of these two particles.

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Purification of β-trypsin by SP-Sephadex column chromatography

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A method for purification of β-trypsin from commercial trypsin is described. The separation was based on the differential retardation of different forms of trypsin by SP-Sephadex column. β-trypsin was identified by SDS-gel electrophoresis.

TRYPSIN undergoes autolysis in solution and results in a number of degraded products. Hence, attempts were made to stabilize this enzyme by simple chemical modification of amino groups[1,2]. The extent of success varied with the type of trypsin used. β-Trypsin could be stabilized when treated with acetimidate whereas α-trypsin failed to stabilize[3]. Commercially available preparations of trypsin differ in the content of aggregated inert material, α-trypsin and β-trypsin[4]. The most commonly used method for separating unautolysed form of trypsin, β-trypsin, from other forms of trypsin is that of Schroeder and Shaw[4] using sulphoxy ethyl-Sephadex C-50 (SE-Sephadex) column. Since Pharmacia Fine Chemicals no longer manufactures SE-Sephadex, efforts were made to purify β-trypsin on sulphoxy propyl-Sephadex (SP-Sephadex) column. The results are reported in this paper. SP-Sephadex C-50 (40–120 μm) from Pharmacia Fine Chemicals, Sweden was used.

The modified method of Schroeder and Shaw[4] for purification of β-trypsin is described below. SP-Sephadex column (1.8 × 80 cm) was equilibrated with tris-HCl buffer (0.01 M, pH 7.0) containing 20 mM Ca^{2+} at 5°C. Trypsin (Sisco Research Laboratory) was dissolved in the above tris-HCl buffer and 10 ml of trypsin solution (10 mg/ml) was loaded on the Sephadex column and eluted using the same buffer. Fractions of 6.2 ml each at a flow rate of 15 ml/h were collected into tubes containing 0.25 ml sodium formate (1.25 M, pH 2.9). Sodium formate was used to immediately lower the pH of the eluted solution thus preventing autolysis of trypsin. Fractions (Nos 105–122, Figure 1) were pooled

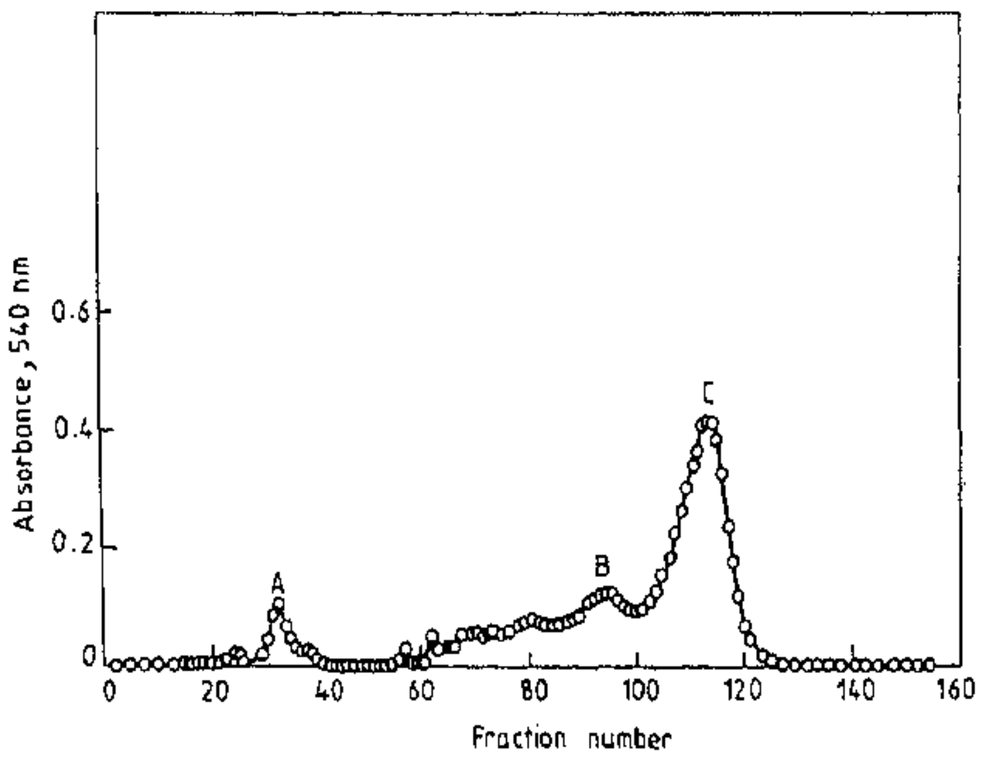

Figure 1. Fractionation of commercial trypsin on SP-Sephadex column.