# CALF ROTAVIRUS: DETECTION OF OUTER CAPSID GLYCOPROTEINS BY LECTINS

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## 1. Introduction

Lectins are known to interact with a variety of enveloped viruses through specific binding to external polysaccharides [1,2]. Until now, no unenveloped viruses, even possessing outer glycoproteins [3,4] have been tested for reactivity with lectins.

Calf rotavirus is provisionally classified in a distinct genus of the family *Reoviridae*. Like other viruses belonging to this family it is unenveloped [5] and possesses a double-stranded RNA genome [6]. When viewed by negative contrast the rotavirus can be distinguished from the *Reovirus* by an outer layer which gives the virus a precise smooth margin [7,8]. It has been also shown that EDTA treatment of the virus leads to removal of this external layer [9,10].

In the present work, we have investigated the presence of glycoproteins on the capsid of calf rotavirus by using the specific affinity of lectins towards carbohydrates.

## 2. Materials and methods

Agglutinins of *Canavalia ensiformis* (Con A), *Triticum vulgare* (WGA), *Phaseolus vulgaris* (PHA-L and PHA-ELs), *Arachis hypogaea* (PNA) and *Phytolacca americana* (Pokeweed mitogene PWM) were from Industrie Biologique, Clichy, France. Lectin of *Limulus polyphemus* (Limulin) was prepared as in (11). Agglutinins of *Ricinus communis* type 1 and type 2 (RcA 1, RcA 2) were from SIGMA.

The procedures for production of calf rotavirus and for purification of dense and light particles have been described [9]. Light particles, referred to as L particles, are fully infectious and have a density of 1.36 g/ml. Dense particles, obtained from L particles through EDTA treatment, are referred to as D particles; the latter are less infectious and have lost the outer protein layer [8,9]. Purified L and D viruses were pelleted and resuspended in 0.1 M NaCl, 1 mM $CaCl_2$, 0.05 M Tris–HCl buffer, pH 7.5. Purity of virus preparation was checked by polyacrylamide gel electrophoresis of proteins and by electron microscopy. The virus concentration was estimated assuming a specific absorbance of 5.4 (concentration 1 mg/ml; $\lambda$ 260 nm; optical path: 1 cm) and assuming $1.13 \times 10^{13}$ particles/mg [12].

The turbidity of virus suspension was measured at $A_{500}$ with a Philips Unicam spectrophotometer [13]. Aggregation of virus particles led to an increase of the turbidity and consequently the $A_{500}$ value. The solutions of lectins and of Me-Man (0.1 M) were added directly to the virus suspensions in the quartz cell.

Tritiated concanavalin A ([$^3$H]Con A) was prepared by the acetylation procedure described [14], using [$^3$H]acetic anhydride (CEA) in the presence of *O*-methyl $\alpha$-D-mannopyranoside to protect the binding sites. [$^3$H]Con A was spec. act. 14 600 cpm/$\mu$g, and, from ultracentrifugation was mol. wt 52 000



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[15]. Con A–virus complexes were separated from free Con A by spinning down the complexes at 160 000 × $g$ (for 10 min) in a Beckman Airfuge. The amount of [$^3$H]Con A specifically bound was calculated by subtracting the amount bound [$^3$H]Con A in the presence of Me-Man from that bound in its absence.

Polyacrylamide gel electrophoresis was performed in slab gels in the presence of sodium dodecylsulfate and mercaptoethanol as in [16]. The gels were washed with Triton-containing buffer to remove the detergent [17], then incubated with the presence of [$^3$H]Con A. Proteins were detected independently with Coomassie blue.

Electron microscopy was done as in [18]; sodium phosphotungstate (4%), at pH 7.4, was used as a negative stain.

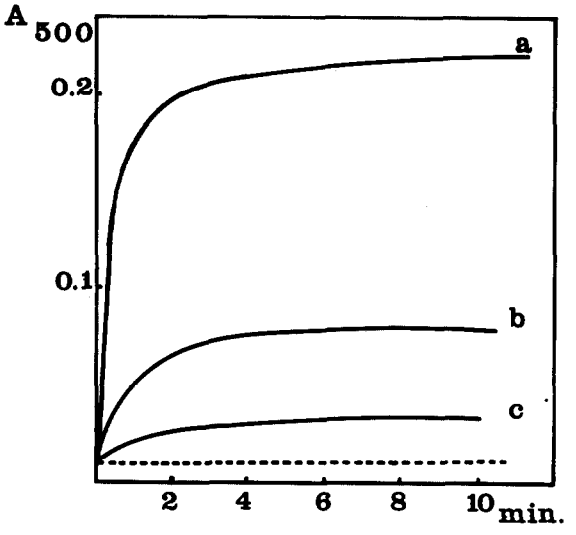



Fig.1. Turbidity of calf L rotavirus suspension as a function of time after addition of various lectins. (——) Effect of the addition of Con A (final conc. 70 μg/ml) to L rotavirus suspension. Virus concentrations were: (a) 300 μg/ml; (b) 70 μg/ml; (c) 25 μg/ml. (– – – –) Turbidity of L rotavirus suspensions (300 μg/ml) after addition of each of the following lectins: PHA-ELs, PHA-Ls, PNA, PWM, RcA 1, RcA 2 (100 μg/ml); WGA (50 μg/ml) and limulin (15 μg/ml).

## 3. Results

The results in table 1 show that Con A was the only one of the lectins tested able to aggregate the L rotavirus. All the others failed to increase the absorbance, whatever the concentration used.

Upon addition of Con A (final conc. 70 μg/ml) to an L rotavirus suspension, $A_{500}$ increased dramatically (fig.1). The absorbance change was very fast, the maximum being reached a few min after lectin addition. The final absorbance depended on the concentration of the virus suspension; a significant increase was detectable with as little as 25 μg/ml virus. The aggregates were easily sedimented (5000 × $g$, 10 min), no detectable absorbance remaining in the supernatant. Aggregation was prevented when $O$-methyl-α-D-mannopyranoside, a Con A-specific ligand, was added to the virus suspension before the lectin. Furthermore, the Con A–virus pellet was easily dissociated on addition of Me-Man, no trace of aggregates remaining within 2 h.

Table 1
Effect of lectins in increasing the absorbance of L rotavirus suspensions

| Lectin | Concentration (μg/ml) | Sugar spec. | L Rotavirus 300 μg/ml |
|---|---|---|---|
| Limulin | 15 | NAN-GalNAc | – |
| PHA-Ls | 10–250 | GalNAc | – |
| PNA | 6–250 | Gal-GalNAc | – |
| PHA-ELs | 10–250 | Gal-GlcNAc-Man | – |
| RcA 1 | 10–150 | Gal | – |
| RcA 2 | 10–150 | Gal | – |
| WGA | 10– 50 | GlcNAc | – |
| PWM | 20–250 | GlcNAc-GlcNAc | – |
| Con A | 10–250 | Man, Glc | +++ |

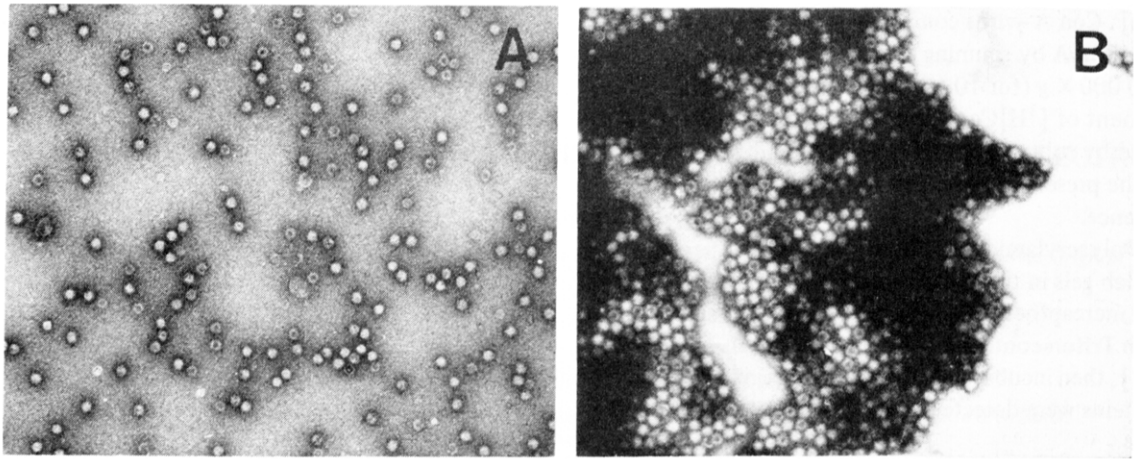



Fig.2. Electron micrograph of calf L rotavirus in the absence (A) and in the presence (B) of Con A. (22 000 ×).

Electron microscopy showed that the virus suspension was not aggregated in the absence of Con A (fig.2A). In the presence of Con A, the virus formed huge aggregates and was tightly associated (fig.2B).

Quantitative data on the binding were obtained using [$^3$H]Con A. Assuming that a virus particle is mol. wt $5.2 \times 10^7$ it can be calculated from the results in fig.3 that a virus particle binds 210 ± 30 Con A molecules, and that the app. $K_a$ is $2.44 \times 10^6$ l × mol$^{-1}$.

When Con A was added to virus pretreated with EDTA, to remove the outer capsid) no increase of absorbance could be detected. Therefore, the D rotavirus particles did not contain the Con A receptors, nor receptors for the other lectins listed in table 1.

## 4. Discussion

The turbidity at $A_{500}$ rotavirus suspension is very low, because the diameter of the particles is only about 65 nm. However, when particles are induced to associate, the turbidity increases, since the light scattering depends on the particle diameter [19]. As with vesicles containing gangliosides [13], addition of a lectin to a suspension of L rotavirus induces a large increase in turbidity. When 30 μg Con A and 70 μg L rotavirus are mixed, the absorbance increased 20 fold, indicating that the aggregates formed are very large.

This point was emphasized by the following results: the aggregates quickly sedimented under a low centrifuge field; no free particles were left in the supernatant; the Con A-virus pellet was seen as dense associated aggregates by electron microscopy. As with vesicles containing gangliosides [13], aggregates were

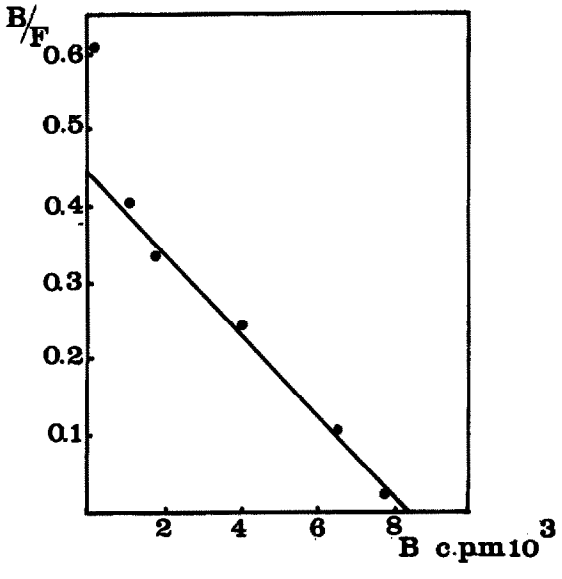
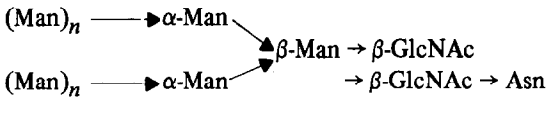



Fig.3. Con A binding to calf L rotavirus. $F$ is the concentration of free Con A in the supernatant after centrifugation, $B$ is the amount of Con A bound to virus particles, expressed as cpm. Virus concentration: 60 μg/ml; total Con A concentrations: 0.4–250 μg/ml.

dissociated by addition of a specific ligand. These results lead to the conclusion that L rotavirus particles contain glycoconjugates in their outer shell, and that these glycoconjugates bind Con A specifically. As evidenced by polyacrylamide gel electrophoresis, in the presence of sodium dodecyl sulfate, the glycoconjugate able to bind [$^3$H]Con A had the same migration as one of the proteins (results not shown). Therefore, it can be concluded that the Con A receptor is a glycoprotein. These findings are consistent with [20].

No glycoconjugates able to bind Con A are present in the inner capsid, since the D rotavirus was not induced to aggregate by the presence of the lectin. The number of Con A-binding glycoconjugates in the outer capsid is quite high, and therefore a large part of the external molecules of the virus must be glycosylated. In contrast, *Reovirus* (Type III) contains only few external glycoconjugates, 10 (–20) of the 550 $\mu_2$-proteins were glycosylated [3].

On the basis of the known specificities [21] of the lectins and the results in table 1, it may be concluded that the outer capsid glycoconjugates of L rotavirus contain either D-glucose or D-mannose residues in terminal non-reducing positions. Furthermore, we may also conclude that these glycoconjugates do not contain D-galactose or *N*-acetyl-D-glucosamine in terminal non-reducing positions since PNA and WGA, which respectively bind these sugars, had no effect. Also, these glycoconjugates do not contain either: NAN → GalNAc which is specific ligand of limulin or (NAN) → Gal → GlcNAc → Man which are specific ligands of RcA and PHA-ELs. Because the glycoconjugates of rotavirus have no affinity for any lectin except Con A, and because glycoproteins have been visualized in polyacrylamide gel electrophoresis, it may be postulated that outer capsid glycoconjugates of calf rotavirus are glycoproteins, and that the sugar moiety could be an oligosaccharide rich in mannose, possibly the following glycopeptide:




with $n$ = 0, 1 or 2, which has been found in several glycoproteins [22] that do not bind any lectin listed in table 1 except Con A. However, further experiments are needed to define the actual nature of the sugar moiety.

In conclusion, the turbidity method appears quite suitable to indicate the presence of sugar in a virus and to give information on the nature of the external sugars. This method may be used with a very small amount of material, only a few $\mu$g virus are required to give a measurable effect. Further, because the aggregation may be easily reversed by a specific ligand, concanavalin A may be used to purify this type of virus.

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