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Effect of Enzymes on Rotavirus Infectivity†

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The infectivity of a bovine rotavirus was enhanced 140-, 8-, and 3-fold, respectively, by trypsin, protease, and lactase. Ficin, carboxypeptidases A and B, lysozyme, and β-galactosidase had little effect on the infectivity. Chymotrypsin caused a threefold decrease in the infectivity. Trypsin acts directly on the rotavirus and not on the host cell.

Previous enzyme studies (9, 13-15) with reoviruses have shown the following: (i) the infectivity of reoviruses can be enhanced by at least eight different proteolytic enzymes; (ii) there are strains of types 1 and 3 reoviruses (called CT^- strains) that are inactivated by chymotrypsin; (iii) the infectivity of the CT^- strains is enhanced by chymotrypsin if ethylenediaminetetraacetic acid (EDTA) is added to the reaction mixture or if the virus is treated in the absence of calcium; and (iv) the enzyme enhances the viral infectivity by removing the outer viral coat.

This study was conducted to determine the effect of enzymes on the reo-like rotaviruses. Unlike the reoviruses, the bovine rotavirus was not enhanced by chymotrypsin or ficin. The slight (three- to sixfold) inactivation of rotavirus by chymotrypsin was not prevented by EDTA. On the contrary, EDTA in the absence of enzyme inactivates the virus. Work in progress suggests that trypsin cleaves a rotavirus polypeptide(s) (A. Carter, B. B. Barnett, and R. S. Spendlove, manuscript in preparation).

Reoviruses and rotaviruses probably both undergo activation as they pass through the gastrointestinal tract. Although a large number of different enzymes will enhance reovirus infectivity, it appears that rotavirus activation is much more specific. In fact, no one has yet determined how to satisfactorily culture a human rotavirus in cell culture. It has been reported that trypsin facilitates the cultivation (1, 2, 5, 10, 16) and plaque assay (8) of bovine, ovine, and porcine rotaviruses.

A reovirus study (15) has shown that viral infectivity is a function of the method of pretreatment of the virus, the viral genome, the host cell used for viral propagation, and the method of assay. A similar thorough study of rotaviruses is needed to facilitate the cultivation of the rotaviruses. This communication reports the initial results of such an investigation.

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A cell culture-passaged vaccine strain of bovine rotavirus obtained from Norden Laboratories, Lincoln, Neb., was grown in Madin-Darby bovine kidney (MDBK) cells and used throughout this study. Infected cell cultures were frozen and thawed three times to release the virus from the cells. The culture fluids were then extracted twice with Freon 113 to remove cell debris. We observed in the immunofluorescent-cell counting procedure (3) that there were many more fluorescent cells when the cultures were infected in the presence of trypsin. To distinguish between effects at the virus and cellular levels, two sets of experiments were performed. In the first, rotavirus was incubated with trypsin (5 μg/ml) for 1 h at 37°C and then a threefold excess of soybean trypsin inhibitor was added to inhibit the trypsin in the virus preparation before infecting the cells. In another experiment, trypsin covalently attached to agarose beads (tolysulfonyl phenylalanyl chloromethyl ketone-trypsin=Sepharose CL-4B; Pierce Chemical Co., Rockford, Ill.) was used to treat the rotavirus, which was then used to infect cells which had not been exposed to trypsin. In the experiment using trypsin inhibitor to prevent exposure of the cells to active trypsin, the rotavirus infectivity was enhanced 20-fold. In the solid-phase trypsin experiment, the rotavirus infectivity was enhanced 50-fold. The results of both experiments indicated that the trypsin enhancement effect is directly on the rotavirus. Trypsin treatment of the cells before infection followed by thorough washing did not result in an increased rotavirus infectivity as compared to nontreated controls.

The effect of a number of enzymes on rotavirus infectivity was then determined. Since enzyme preparations can vary considerably in purity, and units of activity are often expressed differently from one supplier to another, the following information on the enzymes used in these studies is provided.

The α-chymotrypsin was from bovine pan-

crease, 3× crystallized with an activity of 54 U/mg as defined by the supplier, Worthington Biochemical Corp., Freehold, N.J. All other enzymes were obtained from Sigma Chemical Co., Saint Louis, Mo. The grades and activities as listed by Sigma were: trypsin (bovine pancreas) type XI, 8,575 U/mg; protease (bovine pancrease) type 1, 9 U/mg; β-galactosidase (*Escherichia coli*) grade IV, 490 U/mg; lactase from *Saccharomyces fragilis*, 6 U/mg; ficin (fig tree) 2× crystallized, 2 U/mg; carboxypeptidase A (DFP treated type II, crystallized), 57 U/mg; carboxypeptidase A (DFP treated type II, crystallized), 57 U/mg; carboxypeptidase B (DFP treated from hog pancreas), 140 U/mg; lysozyme (egg white) grade 1, 3× crystallized, 20 U/mg. The bovine rotavirus was exposed to enzymes for 1 h at 37°C, diluted 50- to 5,000-fold in Eagle minimum essential medium and assayed by the immunofluorescent-cell counting method (3) (Table 1). There were 140-, 8-, and 3-fold increases in infectivity, respectively, with trypsin, protease, and lactase. The virus preparations exposed to chymotrypsin (at 50 μg/ml) decreased threefold in infectivity. Ficin, β-galactosidase, carboxypeptidases A and B, and lysozyme had little effect on the infectivity.

These results differ from those obtained with reoviruses, i.e., reovirus infectivity can be enhanced by chymotrypsin and ficin as well as by trypsin and protease (14). The infectivity of some reovirus strains is lost after an initial transient enhancement when exposed to proteolytic enzymes if calcium is present (14). Therefore, experiments on the inactivation of rotavirus by chymotrypsin were conducted in the presence and absence of the chelating agent, EDTA. During the first few minutes in the presence of chymotrypsin, there was a three- to sixfold increase in rotavirus infectivity, after which there was a loss of infectivity. Unlike reovirus, rotavirus was not protected by 0.005 M EDTA. Higher concentrations of EDTA inactivated the virus.

Holmes et al. (7) observed complete or partial uncoating of rotavirus by β-galactosidase and lactase; they did not test for infectivity changes. They postulated that lactase in vivo acts as a combined receptor and uncoating enzyme, and that the absence of lactase in cell cultures explains the difficulty encountered in culturing rotaviruses.

Rotaviruses have been exposed to enzymes and examined by electron microscopy. Rodger et al. (12) reported the morphology of bovine rotavirus particles to be unaffected by treatment with chymotrypsin, pepsin, papain, bromelain, or pronase. Infectivity was not determined in these studies. Palmer et al. (11) found that the human rotavirus was morphologically stable when exposed to chymotrypsin, papain, or pepsin, but was completely degraded by a trypsin–EDTA mixture. Almeida et al. (1) examined bovine rotavirus grown in the presence of trypsin; they reported an increased ratio of "smooth" to "rough" particles when trypsin was incorporated in the maintenance media. The harvested particles did not show any signs of degradation in spite of being grown in the presence of trypsin.

Rotavirus RNA polymerase has been shown to be activated by heat shock or treatment with 1 mM EDTA but not by chymotrypsin (250 μg/ml) or pronase (200 μg/ml) (6). This activation was accompanied by a shift in the buoyant density of the virus in CsCl from 1.359 to 1.378 g/ml. It was postulated that this might correspond to the infectious smooth particles (ρ = 1.36 g/ml) and noninfectious rough particles (ρ = 1.38 g/ml) described by Bridger and Woode (4), but no infectivity studies were reported.

By enhancing the infectivity of a bovine rotavirus with trypsin, we routinely produce cell culture fluids with infectivity titers of 5×10^9 fluorescent-cell-forming units/ml. We are currently investigating the mechanism of this proteolytic enhancement of rotavirus infectivity.

TABLE 1. *Rotavirus titers after treatment with enzymes*[a]

Enzyme	Concn (U/mg)	Control (no enzyme)	5 μg of enzyme/ml	50 μg of enzyme/ml
Trypsin	8,575	8.0×10^6	8.0×10^8	1.1×10^9
Protease	9	8.0×10^6	5.0×10^7	6.0×10^7
β-Galactosidase	490	8.0×10^6	1.6×10^7	1.4×10^7
Lactase	6	8.0×10^6	1.6×10^7	2.4×10^7
α-Chymotrypsin	54	8.0×10^6	1.1×10^7	2.7×10^6
Ficin	2	8.0×10^6	8.0×10^6	8.0×10^6
Carboxypeptidase A	57	8.0×10^6	7.2×10^6	7.2×10^6
Carboxypeptidase B	140	8.0×10^6	6.0×10^6	—
Lysozyme	20	8.0×10^6	7.0×10^6	7.0×10^6

[a] Bovine rotavirus was incubated for 1 h at 37°C with the indicated concentration of the enzyme, then assayed by an immunofluorescent cell counting procedure. Results, unless otherwise indicated, are expressed as fluorescent-cell-forming units per milliliter. —, Not done.

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