POLYPEPTIDE STRUCTURE OF BOVINE ENTERITIC CORONAVIRUS:
COMPARISON BETWEEN A WILD STRAIN PURIFIED FROM FECES AND
A HRT 18 CELL ADAPTED STRAIN

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Structural proteins of many of the members of the coronavirusidae have been already studied (for reviews see 1). The variety of their number and size, the variability in the data can be accounted for by the different methods used in purification of the virions, protein dissociation and detection on polyacrylamide gel. Furthermore the origin of the virus from animal or from cell cultures can possibly play a role in the difference observed for the same virus.

For these reasons we compared in the same purification and polyacrylamide SDS gel conditions structural polypeptide patterns of a wild strain of Bovine Enteritic Coronavirus (BECV - Strain ws-) stained by coomassie brilliant blue and of the same HRT 18 cell adapted strain (F15) as revealed by autoradiography.

Virus

BECV-ws was produced on gnotobiotic calf as previously described (2). Diarrheic feces were harvested and clarified, after three fold dilutions by low speed centrifugation. This BECV-ws multiplies spontaneously with very high efficiency in HRT 18 cells ; this line established from human rectum adenocarcinoma presents some properties of the differentiated brush border cell of the intestinal villi. Titers obtained are very high (5×10^7 TCID₅₀/ml virus strain F15). The virus produced had the characteristic of BECV : shape, spikes, density 1.19, hemadsorption and hemagglutination of Rat erythrocytes.

^{14}C aminoacid labelled BECV-F15 was produced in 7 day old HRT 18 cell monolayers maintained in 2 % foetal calf serum RPMI medium containing 2 $\mu\text{Ci/ml}$ ^{14}C protein hydrolysate (C.E.A.) for three days. After freezing and thawing the virus suspension was clarified and titrated (starting material 60 ml at 17×10^7 PFU/ml).

Purification of the virus.

5 ml of virus suspension are layered onto a 20 % to 45 % wt/wt sucrose gradient and centrifuged in a SW 27 Beckman rotor for 90 min at 25,000 rpm, 4°C. The virus band is collected and pelleted after dilution in water (SW 50 Beckman rotor for 90 min., 45,000 rpm, 4°C). The pellet is then resuspended in distilled water and layered onto a 20 % to 60 % wt/wt sucrose gradient and centrifuged in a SW 27 rotor for 17 hrs at 25,000 rpm 4°C.

Fractions are collected and O.D. at 254 nm monitored (Isco U.A. 5 density gradient fractionator). Refractive index are read on Carl Zeiss refractometer, and radioactivity counted.

Polyacrylamide gel electrophoresis of virus polypeptides.

Purified virus fractions from linear sucrose gradients were treated with 3 % sodium dodecyl sulfate, 5 % 2-mercaptoethanol at 100°C for 1.5 min. A trace amount of bromophenol blue dye was added to the reduced polypeptides which were then electrophoresed in slab gels using the discontinuous SDS-gel system of Laemmli (3). After the run the slabs were stained in 0.1 % (wt/vol) Coomassie brilliant blue in acetic acid-methanol-water (5:40:55), analyzed for material absorbing at 620 nm on a Philips densitometer and when necessary processed for autoradiography.

The molecular weights of BECV specific proteins were determined by comparing their electrophoretic mobilities in polyacrylamide gels with proteins of known molecular weights (Calibration proteins for SDS electrophoresis Boehringer) : trypsin inhibitor from soybean (21,500), bovine serum albumin (68,000), RNA polymerase from E. Coli : subunit (39,000), subunit (155,000), subunit (165,000).

RESULTS

The polypeptide patterns obtained for BECV-ws and BECV-F15 are presented in figure 1 a, b.

Figure 1.

It clearly shows that they are very similar in the number of peaks and in polypeptide sizes : 125,000, 65,000, 50,000, 45,000, 36,000, 34,000 and 28,000 daltons. VP₅₀ is the major protein.

From these results we can conclude that this BECV isolate is composed of seven polypeptides, that adaptation to HRT 18 cells does not induce modifications in the structure of the virion and that the same purification and PAGE methods give the same results or by staining of protein bands or by autoradiography. Nevertheless our results are different from those published on Enteropathogenic Bovine Coronavirus Strain Ly 138 (4).

Literature cited:

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