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Biological Properties and Carbohydrate Composition of Human Parainfluenza Virus Type 1 in Two Host Systems

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SUMMARY

Human parainfluenza virus type 1 (HA2 virus) grown either in embryonated hens' eggs or in M. rhesus monkey kidney cells showed differences in size, biological properties and carbohydrate composition. Egg-grown virus showed a larger size (233 nm versus 167 nm), a higher neuraminidase activity (specific activity and initial and maximum velocity) and a higher haemolytic activity than monkey kidney cell-grown virus. The haemagglutinin titre was identical for the HA2 strain grown in both host systems when tested with human O Rh^+, guinea-pig and hen red blood cells, but reduced by more than 100-fold when tested with grivet monkey red blood cells. In addition, the carbohydrate content (mainly neutral sugars) was higher in egg-grown virus (9·2%) than in virus grown in MK cells (5·7%), and the amino to neutral sugar ratio was lower (1·2 versus 2·1). The sugars were identified as fucose, mannose, galactose, glucose, glucosamine and galactosamine. The prominent neutral monosaccharide was glucose in egg-grown virus and fucose in MK cell-grown virus. HA2 virus infection of MK cells increased fucose and glucose, and decreased mannose and galactose levels.

The paramyxoviruses mature by budding through the host cell membrane; they contain 6% carbohydrate which is associated mainly with proteins and lipids (Kingsbury *et al.*, 1978). The glycoproteins are responsible for three different biological activities of the viral envelope. The HN glycoprotein possesses haemagglutinin (HA) and neuraminidase (N) properties (Scheid *et al.*, 1972) and the F glycoprotein possesses haemolysin (HL) activity (Scheid & Choppin, 1974). The carbohydrates associated with lipids in the virion are determined by the host cell (Klenk & Choppin, 1970) and it is thought that this is also true for the carbohydrate side chains of glycoproteins as in the case of other enveloped viruses (Choppin & Compans, 1975).

We have previously shown that HA2 virus contains HA and N activities, the specificities of which have been characterized (Kessler *et al.*, 1977). In the present paper we report that HA, N and HL activities varied when the HA2 virus was grown in different host systems. These different biological properties were associated with changes in the carbohydrate composition of the virions and with a modification in sugar composition of infected MK cells as compared with uninfected cells. These data are in favour of a role of the cells in specifying the carbohydrates of the virions.

HA2 virus, derived from the Chanock strain of human parainfluenza virus type 1 (Chanock *et al.*, 1958), was grown either in primary rhesus monkey kidney cell cultures or in embryonated hens' eggs. Cell-grown virus (MK-HA2) was harvested after 7 days incubation at 33 °C of cells infected with 0·1 or 1 $TCID_{50}$/cell and maintained in 199 medium supplemented with anti-SV5 antiserum to prevent contamination by this virus. Egg-grown virus (E-HA2) was collected from allantoic and amniotic fluids of 8-day-old embryonated hens' eggs inoculated with about 10^4 $TCID_{50}$/egg and incubated for 5 days at 34 °C.

After cellular debris were discarded, HA2 virus was concentrated by ultrafiltration using an Amicon DC2 system according to Kessler & Aymard (1979). After two repeated high and

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Table 1. *Biological characteristics of E- and MK-HA2 viruses*

(*a*) *Neuraminidase activity*

Property	MK-HA2		E-HA2	
	N-lactose	Fetuin	*N*-lactose	Fetuin
Specific activity (mU/mg)	24	1·1	35	7·3
Initial velocity (mU/mg)	80	1·8	94	10·5
Maximum velocity (mU/mg)	265	33	476	95
Optimum pH	5–5·4	5·4	5	5
Optimum temperature (°C)	37–40	ND*	37	ND
$K_m \times 10^{-3}$ M	5	4·1	5	4·1

(*b*) *Haemagglutinin activity* (HAU/mg)

Red blood cells of	MK-HA2	E-HA2
Human O Rh^+	1600	1600
Guinea-pig	800	1600
Hen	1600	3200
Monkey	800	< 10

(*c*) *Haemolytic activity* (HLU50%/mg)

Red blood cells of	MK-HA2	E-HA2
Human O Rh^+	10	225
Guinea-pig	⩽ 10	30
Hen	10	60
Monkey	< 10	< 10

* ND, Not determined.

low speed centrifugations, purification was achieved by three centrifugations through linear sucrose gradients (22 to 60%, w/w) in phosphate-buffered saline (PBS) (Kessler *et al.*, 1977). In this way only a sharp band of viral material was detected. No cellular contaminant was evident in electron microscopic observations. The purity of the preparations was further controlled by polyacrylamide gel electrophoresis of polypeptides (Kessler *et al.*, 1975; Kessler, 1980) and by estimation of residual cellular radioactivity according to Klenk & Choppin (1969) (< 1%).

About 250 particles derived from 10 preparations of purified MK-HA2 and E-HA2 were observed by electron microscopy. Unfixed virions, negatively stained with 1% sodium silicotungstate, were roughly spherical and their surface was covered with spikes (about 12·5 nm). The only significant difference between the two kinds of particles was their size: 167 ± 37 nm with MK-HA2 and 233 ± 66 nm with E-HA2.

The biological activities were compared for about five preparations of virus grown in each host. Results are listed in Table 1. Neuraminidase tests were performed according to Aymard-Henry *et al.* (1973) in 0·1 M-citrate–0·2 M-phosphate buffer pH 5 to 5·4, Two substrates were used: *N*-acetylneuraminlactose (Sigma; 85% of α 2 → 3 bonds and 15% of α 2 → 6 bonds, 1 mg/ml) and fetuin [prepared as described by Han & Puck (1962) at 20 mg/ml]. Neuraminidase activity was expressed as mU/mg protein (= nmol NANA/mn/mg).

Biochemical characteristics of HA2 neuraminidase [optimum pH, optimum temperature and Michaelis constant (K_m) (Table 1*a*)] were similar for both viruses with a particular substrate. The differences were restricted to the initial and maximum enzyme velocity and to the specific activity with fetuin as substrate. Haemagglutinin tests were performed by a microtitre method. A mixture of the specimen to be tested (in 5×10^{-3} M-phosphate–0·15 M-NaCl buffer) and 0·5 to 1% red blood cells from various species (total vol. 0·15 ml) were incubated at room temperature for 1 h in Cooke microtitre plates.

The host system did not modify the specific activity (HAU/mg protein) of HA2 virus haemagglutinin for human, guinea-pig or hen red blood cells. However, the HA titre of E-HA2 virus was at least 100-fold less than that of MK-HA2 virus (Table 1*b*), when tested

with monkey red blood cells. Haemolysin tests were performed as previously described (Kessler *et al.*, 1979) using 0·5 to 1% various red blood cell species. The specific activity of HA2 virus haemolysin was greatly modified by the host system. Indeed, MK-HA2 was unable to haemolyse any species of red blood cells, whereas E-HA2 virus haemolysin was active on human, guinea-pig and hen red blood cells (30 to 225 HLU50%/mg) (Table 1 *c*).

For carbohydrate analysis viruses were dialysed for 3 days against distilled water and then freeze-dried. Neutral sugars were obtained after acid hydrolysis in 1 M-HCl for 3 h at 100 °C, and amino sugars were liberated by 4 M-HCl for 3 h at 100 °C. Neutral carbohydrates were quantified using a colorimetric method with anthrone at 625 nm (Ashwell, 1957), and glucose was employed as a standard. After acetylation, osamines were estimated by the Morgan-Elson reaction according to Reissig *et al.* (1955) using glucosamine as a standard. Overall composition of carbohydrates was estimated on three preparations. Table 2 (*a*) shows that the total carbohydrate content of E-HA2 was higher than that of MK-HA2 virus, and this increase (130%) mainly affected the neutral sugar content, whereas amino sugars were only slightly increased (28%).

Gas–liquid chromatography (GLC) was carried out using an Intersmat IGC 120 FB apparatus equipped with a flame ionization detector. Sugars were analysed by GLC as alditol acetates (Gunner *et al.*, 1961) on a glass column coated with 3% EC NSS-M on gas chrom Q (80 to 100 mesh) (1/4 in × 2 m) at 190 °C with N_2 as carrier gas ($1{\cdot}6 \times 10^5$ Pa) (temperature injector 195 °C, temperature detector 205 °C).

Preliminary attempts were made to determine the optimum conditions for hydrolysis. Fucose analysis requires a mild hydrolysis (1 M-HCl for 1 h); since the drastic conditions (4 M-HCl for 4 h) required for amino sugar bond cleavage destroyed about 50% of the fucose, we chose 2 M-HCl for 2 h at 100 °C. In Table 2 (*b*) data concerning the identification of carbohydrates of HA2 virus (3 to 5 assays) are expressed as molar ratios, calculated from peak areas and as percentages of dry wt. of the virion. The following sugars were detected in both viruses: fucose, mannose, glucose, galactose, glucosamine and galactosamine. Striking differences were observed in the ratios of fucose/glucose and mannose/glucose which were respectively 7-fold and 14-fold higher in MK-HA2 than in E-HA2. While the molar ratio of galactose/glucose was not significantly modified, the percentage of galactose was notably increased in E-HA2 virus (1·5% in E-HA2 virus versus 0·2% in MK-HA2 virus). The same was observed with glucose (2% in E-HA2 virus versus 0·4% in MK-HA2 virus).

The quantitative data obtained by GLC for amino sugars were inferior to those of colorimetric determinations because of the incomplete release of amino sugars during the hydrolysis with 2 M-HCl for 2 h. The molar ratio of galactosamine/glucosamine was lower in E-HA2 virus (1·2) than in MK-HA2 virus (1·8). That is due to an increase of glucosamine in E-HA2 virus (60%) while the amount of galactosamine was not modified.

In order to study the modifications of cell carbohydrate content induced by viral infection, infected (1 $TCID_{50}$/cell) and mock-infected monkey kidney cells were harvested 7 days after infection, washed three times in PBS pH 7·2, suspended in distilled water, then dialysed against distilled water and lyophilized. No significant difference was observed in total carbohydrate content of infected and mock-infected cells (about 0·3% of dry wt.). As shown in Table 2 (*c*) carbohydrates occurring in infected and mock-infected cells were identified as fucose, mannose, galactose, glucose, glucosamine and galactosamine. HA2 virus infection induced an increase of fucose and glucose and a decrease of mannose and galactose. The molar ratio of mannose/fucose fell from 7 in mock-infected cells to 1 in infected cells, while the ratio of glucose/galactose rose from 1·3 to 6·2. For amino sugars, a slight decrease of the galactosamine/glucosamine ratio was observed.

Carbohydrates of enveloped viruses depend on the host cell, as reported for Sendaï virus, NDV and SV5 (Matthews, 1979) and on the virus, which plays an important role in

Table 2. *Carbohydrate composition of purified HA2 virus and HA2-infected M. rhesus monkey kidney cells*

(*a*) *Total carbohydrate content of HA2 virus**

Virus	Total sugar	Neutral sugar	Amino sugar
MK-HA2	5·7	1·8	3·9
E-HA2	9·2	4·2	5

(*b*) *Carbohydrate composition of HA2 virus*

		Neutral sugar†				Amino sugar‡	
Virus		Fucose	Mannose	Galactose	Glucose	Glucosamine	Galactosamine
MK-HA2	Molar ratio	1·70	1·10	0·50	1	1	1·8
	% Dry wt.	0·72	0·45	0·21	0·42	1·4	2·5
E-HA2	Molar ratio	0·23	0·08	0·77	1	1	1·2
	% Dry wt.	0·46	0·16	1·55	2·02	2·3	2·7

(*c*) *Influence of HA2 virus infection on the carbohydrate composition of rhesus monkey kidney cells*

	Neutral sugar†				Amino sugar‡	
Cell	Fucose	Mannose	Galactose	Glucose	Glucosamine	Galactosamine
Mock-infected	0·10	0·70	0·73	1	1	2
Infected	0·23	0·26	0·16	1	1	1·43

* Results are expressed as % dry wt.
† Results are expressed as molar ratio with respect to glucose.
‡ Results are expressed as molar ratio with respect to glucosamine.

determining the carbohydrate moiety of the viral envelope (Nakamura & Compans, 1979). Our data show that the human parainfluenza type 1 virus carbohydrates are mainly dependent on the host system; indeed, they account for 5·7% of MK-HA2 virus dry wt. as in cell-grown SV5 (Klenk & Choppin, 1969), while they rose to 9·2% in E-HA2 virus. The same kind of variation was observed with mumps virus (A. Voiland & E. Lamoure, unpublished observation). We found the same monosaccharides in HA2 virus as in SV5 (Klenk *et al.*, 1970) and in Sendaï virus (Kohama *et al.*, 1978). Like other paramyxoviruses, HA2 virus lacks neuraminic acid (Choppin & Compans, 1975). As we have found changes in biological properties and in sugar content according to the host, we suggest a possible role for carbohydrates in the expression of these activities. The increase in glucose and galactose levels seems to be involved in the stimulation of HA2 virus biological activities. Previous work on fucose localization in glycoproteins using [^{14}C]fucose-labelled MK-HA2 virus shows a 2·5-fold higher incorporation of precursor in HN than in F (Kessler, 1980), and it can be inferred that fucose is associated with haemagglutinin and/or neuraminidase activities.

Further studies will be necessary to define the respective contribution of sugars in glycoproteins and glycolipids. HA2 virus infection of monkey kidney cells produced a significant decrease in mannose and galactose amounts, while a simultaneous increase of fucose and glucose was observed. The metabolic pathway converting GDP-D-mannose to GDP-L-fucose via GDP-4-keto-6-deoxy-D-mannose and GDP-4-keto-6-deoxy-L-glucose could explain the correlated variations of mannose and fucose; likewise, the increase of glucose could proceed from the interconversion of UDP-D-galactose to UDP-D-glucose via UDP-4-keto-D-glucose. A more extensive study would be of interest to define the kinetics of these changes and to elucidate in part the influence of viral replication on infected cell metabolism.

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