Physico-chemical properties of murine hepatitis virus, strain A 59

Brief Report

C. Daniel and P. J. Talbot

Centre de recherche en virologie, Institut Armand-Frappier,
Université du Québec, Laval-des-Rapides, Laval, Québec, Canada

Accepted June 15, 1987

Summary

The infectivity of murine hepatitis virus (MHV-A 59) was optimally stable at pH 6.0 and was unaffected by ionic strength or at least 15 cycles of freezing and thawing. It was completely inactivated within 25 minutes at 56°C, but was protected by 1 M magnesium chloride or magnesium sulphate. It was completely inactivated within 14 days at 37 and 22°C, but was relatively stable for as long as 72 days at 4°C and optimal pH.

*

Murine hepatitis viruses (MHV), important members of the Coronaviridae family (25), are responsible for several diseases of rodents, in particular respiratory, neurological and gastrointestinal disorders (24, 34). They are characterized by club-shaped surface projections, about 20 nm in length and form pleomorphic virions of 80 to 120 nm in diameter; they possess single stranded RNA of positive polarity and a lipid containing envelope (23, 25, 28).

Physico-chemical properties of some coronaviruses have been described by several authors. These studies showed that pH and temperature stabilities were variable among different members of the coronavirus group, according to the strains used. For instance, some strains of the avian infectious bronchitis virus (IBV) were stable to acid pH whereas others were labile (1, 6, 7, 8, 26). On the other hand, transmissible gastroenteritis virus (TGEV) has been reported to be stable for at least one hour at 37°C between pH 4 and 8 (18). However, this virus showed marked instability at pH above its optimum pH of 6.5, when incubated for 24 hours (21). Similar to IBV, TGEV strains showed great variability in their stabilities to an acidic environment (12). Moreover, TGEV strains were generally heat-labile since they were inactivated in a few days at 37°C and in 10 minutes at 56°C (3, 15, 18).

Interestingly, Coria (5) reported that 1 M magnesium sulphate could protect IBV from thermal inactivation at pH values ranging from 4 to 10 whereas Cowen et al. (7) found that this virus was inactivated at high temperature (50–56°C) with or without 1 M magnesium chloride. Finally, sialodacryoadenitis virus (SDAV), a possible member of the coronavirus group (17, 24), was characterized by Bhatt et al. (2), who found it to be relatively acid-resistant and heat-labile.

Some physico-chemical properties of strains JHM, 2 and A 59 of the murine hepatitis virus group have been described. Cheever et al. (4) observed rapid inactivation of MHV-JHM at 56°C, persistence of infectivity at 37°C for 3 hours and a slow inactivation at 4°C. Manaker et al. (16) first isolated the A 59 strain and reported properties similar to those of JHM. Sturman (27) reported on the pH dependence of MHV-A 59 infectivity. At 37°C, virus infectivity was most stable between pH 6.0 and 6.5 whereas at 4°C, virus was stable over the range of pH 4 to 8. Hirano et al. (14) determined that MHV-2 was heat-labile but was protected from inactivation at 56°C by 1 M magnesium chloride or 1 M magnesium sulphate. This virus was also stable at pH 3 to 9 at 37°C for 60 minutes. In summary, it appears that physico-chemical properties differ within the coronavirus group according to the experimental conditions and the intrinsic properties of the virions. In the present study, we report on some of the physico-chemical properties of MHV-A 59 grown on DBT cells.

Murine hepatitis virus (MHV), strain A 59, was obtained from the American Type Culture Collection (Rockville, MD, U.S.A.), plaque purified twice and passaged four times on DBT cells prior to use as inoculum in all experiments described. The DBT murine cell line (13) was kindly supplied by Dr. Michael J. Buchmeier (Scripps Clinic and Research Foundation, La Jolla, CA, U.S.A.) and grown at 37°C in Earle's minimum essential medium/Hank's M 199 (1:1, v/v) supplemented with 5 percent (v/v) foetal calf serum (FCS) and 0.13 percent (w/v) sodium bicarbonate (Gibco Canada, Burlington, Ontario). Plaque assay of MHV-A 59 was carried out by a modification of the method reported by Hirano et al. (13) in 35 mm diameter tissue culture 6-well cluster plates (Flow Laboratories, Mississauga, Ontario, Canada). All samples were homogenized by vortexing and ultra-sonication for 5 to 10 seconds in order to eliminate possible virus aggregates before dilution and inoculation. After one hour adsorption at 37°C on a rocker platform, the virus inocula were removed, the monolayers overlaid with medium containing 1.5 percent (w/v) bacto-agar (Difco Laboratories, Detroit, MI, U.S.A.), 0.05 mg/ml gentamycin and 10 percent (v/v) FCS. Plates were incubated for 48 to 72 hours at 37°C, fixed with 9.25 percent (v/v) formaldehyde and stained with crystal violet.

Virus was incubated in buffers of various pHs for 6 hours at 37 or 4°C and residual infectivity was titrated. The results are shown in Fig. 1. We

found that MHV-A 59 was stable over a wide pH range (3.0–10.0) at lower temperature (4°C), whereas at 37°C, it was only stable between pH 5.0 and 7.4. Thus compared with MHV-2 (14) and MHV-3 (20), MHV-A 59 was more sensitive to pH, particularly at 37°C. A similar relationship shown between temperature and pH sensitivity was also observed with other coronaviruses such as TGEV (21) and IBV (1) and it has been suggested that pH-dependent thermolability is due to aggregation of the E 2 peplomeric glycoprotein (27). MHV-A 59 was most stable at pH 6.0, as described for IBV and TGEV (29) and in extension of a report by Sturman (27).

The pH sensitivity test described in Fig. 1 was also performed in buffers without sodium chloride, with similar results (data not shown). The sensitivity of MHV-A 59 to ionic strength was further investigated with sodium chloride concentrations of 0 to 500 mM added to solutions of 10 mM Tris acid maleate pH 6.0, containing 5 percent (v/v) FCS. Ten-fold dilutions of virus in these buffers were incubated at 4 or 37°C for 6 hours and stored at –70°C

Fig. 1. Effect of pH on MHV-A 59 infectivity. Virus samples were incubated from pH 3 to 8 (0.1 M citric acid and 0.2 M sodium phosphate dibasic) and pH 9 to 12 (0.1 M glycine in 0.1 M sodium chloride and 0.1 N sodium hydroxide), with 150 mM sodium chloride and 5 percent (v/v) FCS, for 6 hours at 4°C (●) or 37°C (■) and frozen at –70°C until assayed for viral infectivity, as described in the text

Fig. 2. Effect of magnesium ions on MHV-A 59 thermal inactivation. Ten-fold dilutions of virus in 0.1 M Tris acid maleate [5 percent (v/v) FCS, pH 6.0] with or without (○) 1 M magnesium chloride (●) or magnesium sulphate (■) were incubated at 56°C for various times and frozen at -70°C until assayed for viral infectivity as described in the text

until assayed for virus infectivity. Results showed that 0 to 500 mM sodium chloride did not affect virus stability at either 37 or 4°C (data not shown). On the other hand, Wallis and Melnick (30, 31) found that 2 M sodium ions (Na⁺) enhanced inactivation of enteroviruses (non-enveloped RNA viruses) at 37°C whereas it protected them from inactivation at 50°C. Furthermore, Na⁺ conferred some protection to vaccinia and adenoviruses from heat inactivation (32).

As shown in Fig. 2, the A 59 strain was rapidly (25 minutes) inactivated at 56°C. It was significantly protected by 1 M magnesium chloride for at least 30 minutes, and slightly more by 1 M magnesium sulphate. Heat stability has not been associated with morphological characteristics such as the presence of an envelope or nucleic acid type. Indeed, poxviruses, papovaviruses and reoviruses were relatively stable when incubated at 50°C for 30 minutes whereas herpes simplex viruses, adenoviruses, enteroviruses and myxoviruses were labile in similar conditions (10). Nevertheless, most corona-

viruses were reported to be inactivated within 10 to 30 minutes at 56°C as we have shown for MHV-A 59 (19, 20, 29). The effect of divalent cations, such as magnesium, has been shown to vary according to the virus and the salt used. Wallis et al. (33) showed that stabilization of polioviruses by salts could not be generalized to cations or anions. Indeed, 1 M magnesium chloride stabilized entero- and reoviruses whereas 1 M magnesium sulphate did not stabilize enteroviruses and enhanced inactivation of reoviruses (31, 33). In addition, 1 M magnesium chloride enhanced inactivation of adeno-, papova-, herpes-, myxo- and poxviruses whereas myxoviruses were the only one stabilized by 1 M magnesium sulphate (22, 33). In the coronaviruses family, TGEV was not protected by 1 M magnesium chloride at 50°C for 60 minutes (18) whereas IBV was protected for 80 minutes at 50°C by 1 M magnesium sulphate (5). On the other hand, Hirano et al. (14) found that MHV-2 in 1 M magnesium chloride or sulphate was not inactivated after heating at 50°C for 15 minutes, results similar to those obtained in our studies on MHV-A 59.

Fig. 3. Kinetics of MHV-A 59 thermal inactivation. Virus was diluted 10-fold in buffer at pH 6.0 as described in the legend to Fig. 1 (except that sodium chloride concentration was 50 mM). Samples were incubated at 4 (○), 22 (●) or 37°C (■) for various times and frozen at -70°C until assayed for viral infectivity as described in the text

The kinetics of virus inactivation at 4, 22 and 37°C were studied and the results are presented in Fig. 3. The virus was relatively stable for 3 months at 4°C whereas infectivity was unmeasurable after 14 days at 22 and 37°C (<200 PFU/ml). The 37 and 4°C samples were kept in the dark but the 22°C sample was in room light and might have increased the inactivation rate at 22°C above that at 37°C. Manaker et al. (16) observed that the infectivity titre of the A 59 strain was not altered after 7 months at 4°C. Other authors described coronaviruses as slowly inactivated at 37°C and moderately stable at 4°C in optimal suspending medium (19, 29). On the other hand, some TGEV strains were inactivated in less than 4 days at 37°C (11, 18) and MHV-2 showed a 400-fold decrease in infectivity after 24 hours at 37°C (14). However, it is not clear whether the other components of the suspending fluid were comparable in these experiments.

Finally, one ml aliquots of virus in growth medium were subjected to cycles of thawing in a 37°C water bath, followed immediately by freezing for at least 2 hours at -70°C. The infectious titre was stable for at least 15 such cycles (data not shown) as was found for several murine hepatitis viruses (19). For instance, MHV-3 resisted six cycles (20), MHV-2 resisted ten (14) and MHV-A 59 six (16).

The physico-chemical properties of MHV-A 59 reported in this study show that these varied between strains though they were morphologically similar and confirm and extend previous reports on the subject. The stabilization of A 59 by magnesium ions had not been reported previously. These results may also be of practical value in selecting conditions for viral production or purification.

Acknowledgements

We thank Francine Lambert and Francine Allard for excellent technical assistance and Lucie Summerside and Lise Brady for typing the manuscript. The work was supported by grants from the Natural Sciences and Engineering Research Council of Canada (U 0387) and the Fonds de la recherche en santé du Québec (840491). A summer studentship award to C.D. from Employment and Immigration Canada is gratefully acknowledged. P.J.T. is recipient of a scholarship from the Natural Sciences and Engineering Research Council of Canada.

References

1. Alexander DJ, Collins MS (1975) Effect of pH on the growth and cytopathogenicity of avian infectious bronchitis virus in chick kidney cells. *Arch Virol* 49: 339-348
2. Bhatt PN, Percy DH, Jonas AM (1972) Characterization of the virus of sialodacryoadenitis of rats. *J Infect Dis* 126: 123-130
3. Cartwright FS, Harris HM, Blandfors TB (1965) A cytopathic virus causing a transmissible gastroenteritis in swine. *J Comp Pathol* 75: 387-396
4. Cheever FS, Daniels JB, Pappenheimer AM, Bailey OT (1949) A murine virus (JHM) causing disseminated encephalomyelitis with extensive destruction of myelin. I. Isolation and biologic properties of the virus. *J Exp Med* 90: 181-194

5. Coria MF (1972) Stabilizing effect of magnesium sulfate on avian infectious bronchitis virus propagated in chicken embryo kidney cells. *Appl Microbiol* 23: 281–284
6. Cowen BS, Hitchner SB (1975) pH stability studies with avian infectious bronchitis virus (coronavirus) strains. *J Virol* 15: 430–432
7. Cowen BS, Hitchner SB, Ubertini T (1971) Characterization of a new infectious bronchitis virus isolate. II. Some chemical and physical properties of Clark 333. *Avian Dis* 15: 527–532
8. Cunningham CH, Stuart HO (1947) The pH stability of the virus of infectious bronchitis of chickens. *Cornell Vet* 37: 99–103
9. Fasman GD (1976) Buffer solutions. In: Fasman GD (ed) *Handbook of biochemistry and molecular biology*, 3rd edn. CRC Press, Cleveland, Ohio, p 362
10. Hamparian VV, Hileman MR, Ketler A (1963) Contributions to characterization and classification of animal viruses. *Proc Soc Exp Biol Med* 112: 1040–1050
11. Harada K, Kaji T, Kumagai T, Sasahara J (1968) Studies on transmissible gastroenteritis in pigs. IV. Physicochemical and biological properties of TGE virus. *Natl Inst Anim Health Q* 8: 140–147
12. Hess RG, Bachmann PA (1976) In vitro differentiation and pH sensitivity of field and cell culture-attenuated strains of transmissible gastroenteritis virus. *Infect Immun* 13: 1642–1646
13. Hirano N, Fujiwara K, Hino S, Matumoto M (1974) Replication and plaque formation of mouse hepatitis virus (MHV-2) in mouse cell line DBT culture. *Arch Ges Virusforsch* 44: 298–302
14. Hirano N, Hino S, Fujiwara K (1978) Physico-chemical properties of mouse hepatitis virus (MHV-2) grown on DBT cell culture. *Microbiol Immunol* 22: 377–390
15. Laude H (1981) Thermal inactivation studies of coronavirus, transmissible gastroenteritis virus. *J Gen Virol* 56: 235–240
16. Manaker RA, Piczac CV, Miller AA, Stanton MF (1961) A hepatitis virus complicating studies with mouse leukemia. *J Natl Cancer Inst* 27: 27–51
17. Maru M, Sato K (1982) Characterization of a coronavirus isolated from rats with sialoadenitis. *Arch Virol* 73: 33–43
18. McClurkin AW, Norman JO (1966) Studies on transmissible gastroenteritis of swine. II. Selected characteristics of a cytopathogenic virus common to five isolates from transmissible gastroenteritis. *Can J Comp Med* 30: 190–198
19. McIntosh K (1974) Coronaviruses: a comparative review. *Curr Top Microbiol Immunol* 63: 85–129
20. Piazza M (1969) *Experimental viral hepatitis*. CC Thomas, Springfield, Illinois, pp 17–116
21. Pocock DH, Garwes DJ (1975) The influence of pH on the growth and stability of transmissible gastroenteritis virus in vitro. *Arch Virol* 49: 239–247
22. Rapp F, Butel JS, Wallis C (1965) Protection of measles virus by sulfate ions against thermal inactivation. *J Bacteriol* 90: 132–135
23. Siddell S, Wege H, ter Meulen V (1982) The structure and replication of coronaviruses. *Curr Top Microbiol Immunol* 99: 131–163
24. Siddell S, Wege H, ter Meulen V (1983) The biology of coronaviruses. *J Gen Virol* 64: 761–776
25. Siddell SG, Anderson R, Cavanagh D, Fujiwara K, Klenk HD, MacNaughton MR, Pensaert M, Stohlman SA, Sturman L, van der Zeijst BAM (1983) Coronaviridae. *Intervirology* 20: 181–189
26. Stinski MF, Cunningham CH (1969) Neutralizing antibody complex of infectious bronchitis virus. *J Immunol* 102: 720–727

27. Sturman LS (1981) The structure and behavior of coronavirus A 59 glycoproteins. In: ter Meulen V, Siddell S, Wege H (eds) *Biochemistry and biology of coronaviruses*. Plenum Press, New York, pp 1–17
28. Sturman LS, Holmes KV (1983) The molecular biology of coronaviruses. *Adv Virus Res* 28: 35–112
29. Tyrrell DAJ, Alexander DJ, Almeida JD, Cunningham CH, Easterday BC, Garwes DJ, Hierholzer JC, Kapikian A, MacNaughton MR, McIntosh K (1978) *Coronaviridae*: second report. *Intervirology* 10: 321–328
30. Wallis C, Melnick JL (1961) Stabilization of polioviruses by cations. *Tex Rept Biol Med* 19: 683–700
31. Wallis C, Melnick JL (1962) Cationic stabilization – a new property of enteroviruses. *Virology* 16: 504–506
32. Wallis C, Yang C, Melnick JL (1962) Effect of cations on thermal inactivation of vaccinia, herpes simplex and adenoviruses. *J Immunol* 89: 41–46
33. Wallis C, Melnick JL, Rapp F (1965) Different effects of $MgCl_2$ and $MgSO_4$ on the thermostability of viruses. *Virology* 26: 694–699
34. Wege H, Siddell S, ter Meulen V (1982) The biology and pathogenesis of coronaviruses. *Curr Top Microbiol Immunol* 99: 165–200

Authors' address: Dr. P. J. Talbot, Centre de recherche en virologie, Institut Armand-Frappier, Université de Québec, 531, boulevard des Prairies, Laval-des-Rapides, Laval, Québec H7N 4Z3, Canada.

Received March 27, 1987