THE INHIBITORY EFFECTS OF MgSO₄ ON THE MULTIPLICATION AND TRANSCRIPTION OF MOUSE HEPATITIS VIRUS

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Abstract

The multiplication of mouse hepatitis virus (MHV) was inhibited by the treatment of infected cells with MgSO₄ at concentrations higher than 50 mM. The inhibition of viral multiplication was more effective with the treatment of cells at the early stage of infection using MgSO₄ than at the late stage. Viral adsorption to the cells was not inhibited by MgSO₄ and pretreatment of the cells with MgSO₄ did not show an inhibitory effect on the RNA synthesis of MHV. The synthesis of viral RNA was inhibited more effectively by the treatment of infected cells with MgSO₄ at 0–2 and 2–4 h postinfection (p.i.) than at 4–6 h p.i. The present study suggests that the stage at which viral multiplication is susceptible to MgSO₄ may be the early stage of viral transcription and that Mg^{2+} may be a useful tool for the analysis of the early stage of MHV infection.

Key Words: MHV, Inhibition, viral multiplication, RNA synthesis, MgSO₄.

Introduction

Mouse hepatitis virus (MHV) is a member of the *Coronaviridae*, which cause a variety of diseases including hepatitis and encephalomyelitis in laboratory mice[5,20]. MHV is an enveloped virus containing a helical nuclecapsid structure composed of a single-stranded, positive-polarity RNA approximately 30 kilobases (kb) in length[12]. During infection, virion RNA is initially translated into RNA-dependent RNA polymerase and transcribed into a full-length negative-stranded RNA by the RNA-dependent RNA polymerase[3,8]. In turn, the negative-stranded RNA is transcribed into a genomic RNA and six to eight species of subgenomic mRNAs[8,14]. The mRNAs form a 3′-coterminal nested-set extending for different lengths in the 5′ direction[8]. The 5′-ends of each mRNA and the genomic mRNA contain an identical leader

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sequence of approximately 70 nucleotides, which are encoded only at the 5'-end of genomic RNA[9,18]. The free leader RNA is synthesized initially, dissociates from the negative-stranded template, and rebinds to the full-length negative-stranded RNA at the initiation sites of 6 to 8 subgenomic mRNAs. The leader RNA thus takes part in a leader-primed transcription[2]. RNA-dependent RNA polymerase plays an important role in the MHV replication cycle. However, the character of MHV RNA polymerase remains unclear. In the course of analyses of the effects of divalent cations on RNA synthesis of MHV, we found that $MgSO_4$ inhibited the multiplication of MHV. In the present study we investigated the effects of $MgSO_4$ on the multiplication and the synthesis of MHV.

MATERIALS AND METHODS

Cell, virus and cultivation : The JHM strain of MHV[10] was used in this study. DBT cells[6] were cultivated in Eagle's minimum essential medium (MEM) supplemented with 5% calf serum (CS) at 37°C in a humidified atmosphere containing 5% CO_2.

Treatment of cells with $MgSO_4$: DBT cells were adsorped with MHV at a multiplicity of infection (m.o.i.) of 1.0 for 1 h at 37°C. After the incubation, the cells were washed twice with CS-free MEM and incubated in MEM with CS for several hours at 37°C. $MgSO_4$ in MEM was added to the culture medium at final concentrations from 50 to 200 mM at various times postinfection. After incubation for several hours at 37°C, the medium containing $MgSO_4$ was removed and then cells were washed 3 times with CS-free MEM. After the addition of MEM with CS, plaque assays were performed to titrate infectious progeny at each time postinfection (p.i.) according to the method of Hirano et al.[6].

Northern blot hybridization : Cellular RNA was prepared from MHV-infected DBT cells treated with $MgSO_4$ according to the method of Silver *et al.*[16]. The RNA samples were electrophoresed in 1% agarose gels containing formaldehyde, and blotted onto nitrocellulose membranes[11]. A cDNA of MHV mRNA 7, which was kindly provided by Dr. Siddell[17], was ^{32}P-labeled by nick-translation[13] as a probe. Prehybridization was carried out for 2 h at 45°C in 25 mM potassium phosphate (pH 7.4), 5× SSC (1×SSC is 0.15 M NaCl and 0.015 M sodium citrate), 5×Denhardt's solution[4] (1 ×Denhardt's solution is 0.05% bovine serum albumin, 0.05% Ficoll and 0.05% polyvinylpyrrolidone), 0.5% SDS, 100 μg/ml of yeast tRNA and 50% formamide. Hybridization was carried out at 45°C for 15 h in fresh hybridization solution containing ^{32}P-labeled cDNA of MHV-mRNA 7. Autoradiography using Fuji RX X-ray film (Fuji Photo Film Co.) was carried out with an intensifying screen at −70°C.

RESULTS

Inhibition of the multiplication of MHV by $MgSO_4$: The DBT cells infected with

MHV strain JHM were incubated in the presence of $MgSO_4$ at concentrations from 50 to 200 mM during 0 to 2 h p.i. at 37°C and plaque assays were performed at 9 h p.i. (Table 1). The yields of infectious virion particles from the cells treated with $MgSO_4$ at concentrations of 50 and 100 mM were significantly reduced, compared with the yields from untreated cells. No progeny virus was observed at 150 and 200 mM. To investigate the inhibitory effects of $MgSO_4$ on MHV multiplication in the replication cycle, the DBT cells infected with JHM strain MHV were treated with 100 mM $MgSO_4$ at 0-2, 2-4 and 4-6 h p.i. and plaque assays were performed at 7, 9.5 and 12 h p.i. (Table 2). No progeny virus from treated cells was observed at 7 h p.i. The yield of the virus from cells treated with $MgSO_4$ at 0-2 h p.i. was significantly lower than those from untreated cells and treated cells at 2-4 and 4-6 h p.i. at 9.5 h p.i. At 12 h p.i., the yield of the virus from treated cells was significantly lower than those from untreated cells, but no significant difference in the yields was observed among the treated cells during 0-2, 2-4 and 4-6 h p.i.

Table 1. Concentration-dependent inhibitory effect of $MgSO_4$ on MHV multiplication.

Concentration of $MgSO_4$ (mM)	PFU/ml at 9 h p.i.
0	2.2×10^4
50	5.6×10^3
100	2.7×10^2
150	0
200	0

Table 2. Inhibition of multiplication of MHV by $MgSO_4$.

Treatment of $MgSO_4$	Yields of MHV (PFU/ml)		
	7 h p.i.	9.5 h p.i.	12 h p.i.
untreated	1.9×10^2	3.8×10^4	2.1×10^5
0 − 2 h p.i.	0	4.9×10^2	6.8×10^4
2 − 4 h p.i.	0	1.3×10^3	6.6×10^4
4 − 6 h p.i.	0	1.5×10^3	9.8×10^4

Effect of $MgSO_4$ on the synthesis of viral mRNA : To investigate the effect of $MgSO_4$ on the synthesis of viral mRNA, cellular RNA was prepared from MHV-infected cells treated with $MgSO_4$ at 100 mM at 6 h p.i. and analyzed by Northern blot hybridization using cDNA of mRNA 7 of MHV as a probe (Fig. 1). Since genomic

1 2 3 4 5 6
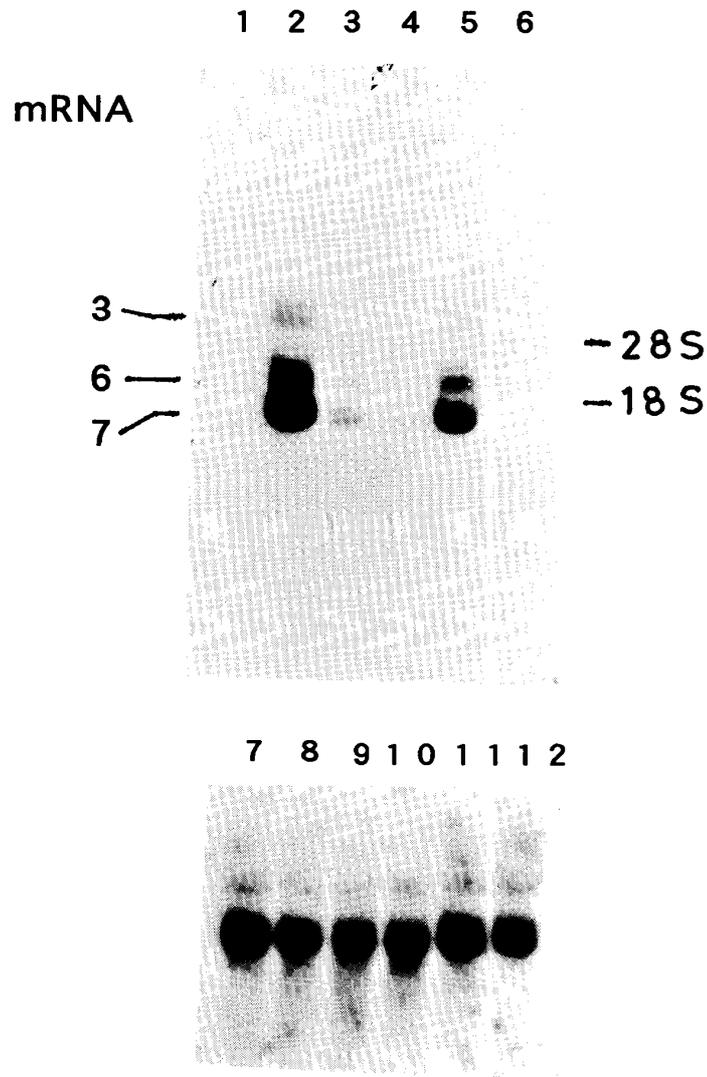
mRNA

3 —

6 —

7 —

— 28 S

— 18 S

7 8 9 10 11 12

Fig. 1. Effects of treatment of MgSO₄ on the RNA synthesis of MHV.

Cellular RNAs were extracted from mock-infected cells (lanes 1 and 7), untreated infected cells (1.0 m.o.i., lanes 2 and 8) and infected cells (1.0 m.o.i.) treated with 100 mM MgSO₄ at 0–2 h p.i. (lanes 3 and 9), 2–4 h p.i. (lanes 4 and 10) and 4–6 h p. i. (lanes 5 and 11) at 6 h p.i. Cellular RNAs were extracted from untreated infected cells (1.0 m.o.i.) at 4 h p.i. (lanes 6 and 12). The RNA samples were analyzed by Northern blot hybridization using cDNA of MHV-mRNA 7 as a probe (lanes 1 to 6). Lanes 7 to 12 were rehybridized with β-actin cDNA as a probe after dehybridization of the filter. mRNA3, 6 and 7 show subgenomic mRNA3, 6 and 7 of MHV, respectively. 18S and 28S rRNAs were used as size markers.
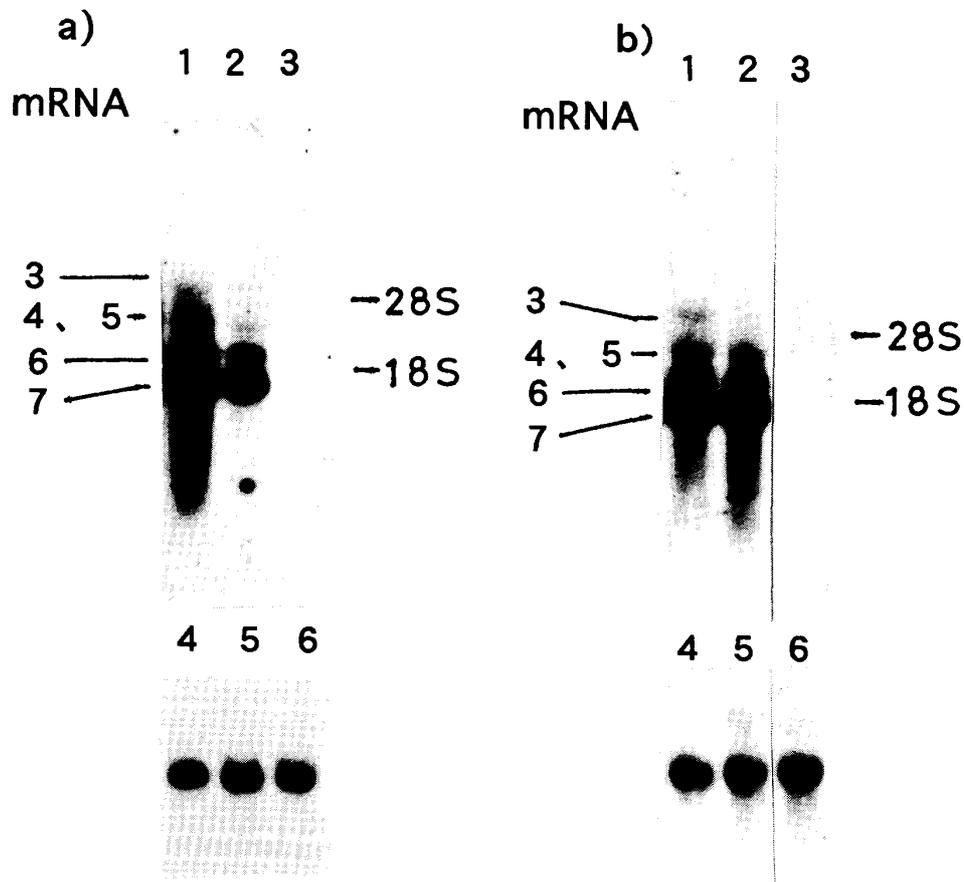
Fig. 2. Northern blot analysis of viral RNA from cells pretreated with MgSO₄ and treated cells incuedted at 4 °C in the presence of MgSO₄.

(a) DBT cells were incubated in the presence of 100 mM MgSO₄ for 2 h at 37°C and infected with MHV (1.0 m.o.i.). The cellular RNAs were extracted from untreated cells (lanes 1 and 4), pretreated cells (lanes 2 and 5) and mock-infected cells (lanes 3 and 6), and analyzed by Northern blot hybridization. Lanes 1 to 3 were hybridized with cDNA of MHV-mRNA 7 as a probe. Lanes 4 to 6 were rehybridized with β -actin cDNA as a probe after dehybridization of the filter.

(b) Cellular RNAs extracted from infected cells (1.0 m.o.i.) which were incubated for 1 h at 4°C in the absence (lanes 1 and 4) or in the presence of 100 mM MgSO₄ (lanes 2 and 5) and mock-infected cells (lanes 3 and 6), and analyzed by Northern blot hybridization using cDNA of MHV-mRNA 7 as a probe (lanes 1 to 3). Lanes 4 to 6 were rehybridized with β -actin cDNA as a probe after dehybridization of the filter. mRNA3, 4, 5, 6 and 7 show subgenomic mRNA3, 4, 5, 6 and 7 of MHV, respectively. 18S and 28S rRNAs were used as size markers.

RNA and subgenomic mRNAs contain a sequence complementary to mRNA 7[9], all mRNA species hybridize with cDNA of mRNA 7. The synthesis of viral mRNAs in the cells treated with $MgSO_4$ was reduced compared to that in untreated cells. Viral-specific bands from the treated cells during 0–2 and 2–4 h p.i. were scarcely observed. In contrast, substantial amounts of viral RNA were observed in the treated cells at 4–6 h p.i. Since no viral RNA was observed in the untreated cells at 4 h p.i. under the conditions used in the present study (Fig. 1, lane 6), these results suggested that viral RNA was synthesized in the presence of $MgSO_4$ at 4–6 h p.i.

Effects of MgSO₄ on early stages of MHV infection : To investigate the effect of pretreatment of the cells with $MgSO_4$ on the multiplication of MHV, the cells were treated with 100 mM $MgSO_4$ for 2 h at 37°C, washed 3 times with CS-free MEM, and then infected with MHV (1.0 m.o.i.). When cellular RNA was extracted at 5 h p.i. and analyzed by Northern blot hybridization using cDNA of mRNA7 as a probe, no difference was observed in viral RNA synthesis between pretreated and untreated cells (Fig. 2a). At 4°C virus particles adsorbs the cellular receptor but does not penetrate into the cells. To investigate the effect of $MgSO_4$ on the virus adsorption, the DBT cells were infected with MHV at 1.0 m.o.i. and incubated at 4°C for 1 h in the absence or presence of 100 mM $MgSO_4$, washed with CS-free MEM and then incubated at 37°C. Cellular RNA was extracted at 5 h p.i. and analyzed by Northern blot hybridization (Fig. 2b). Substantial viral RNA was observed in the cells incubated at 4°C in the presence of $MgSO_4$. This result suggested that viral adsorption was not inhibited by $MgSO_4$.

DISCUSSION

The present results showed that treatment of infected cells with $MgSO_4$ inhibited MHV multiplication at concentrations higher than 50 mM. The inhibition of viral multiplication was more effective with the treatment of cells in the early stage of infection with $MgSO_4$ than in the late stage (Table 2). Since pretreatment of the cells with $MgSO_4$ did not inhibit the viral RNA synthesis (Fig. 2b), it is suggested that the cellular receptor to MHV is not irreversibly affected by $MgSO_4$. Furthermore, viral adsorption was not inhibited by $MgSO_4$ (Fig. 2a). Treatment of virus particles with $MgSO_4$ did not affect the viral infectivity (data not shown and Hirano et al.[7]). Therefore, the inhibition of MHV multiplication was not due to the direct effect of $MgSO_4$ on the virus particle. The synthesis of viral RNA was inhibited by treatment of the cells at 0–2 h and 2–4 h p.i., and viral RNA was synthesized in the presence of $MgSO_4$ at 4–6 h p.i. (Fig. 1). Therefore, the stage susceptible to $MgSO_4$ seems to be the early stage of viral transcription.

Although the details of the character of the RNA-dependent RNA polymerase of MHV remain unclear as yet, a high concentration of Mg^{2+} inhibits the RNA synthesis of Japanese encephalitis virus *in vitro*[19]. Therefore, RNA-dependent RNA

polymerase of MHV may be inhibited by a high concentration of Mg^{2+}. The MHV genome is a single-stranded $RNA^{12)}$. It is suggested that the single-stranded RNA forms a secondary structure and that the formation and flexibility of the secondary structure of MHV RNA may play an important role in RNA synthesis[1,15). Since Mg^{2+} affects the stability of the secondary structure of single-stranded RNA, a high concentration of Mg^{2+} may fix the secondary structure of viral RNA, resulting in the inhibition of RNA synthesis. Furthermore, the fixed secondary structure of viral RNA may affect the synthesis of RNA-dependent RNA polymerase in the early stage of the viral replication cycle.

The early events of the replication cycle of MHV and the interaction of genomic RNA-dependent RNA polymerase remain, however, unclear. The present study suggests that Mg^{2+} may be a useful tool for the analysis of such events.

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