Arch Pharm Res Vol 23, No 3, 257-260, 2000

Evaluation of tTA-Mediated Gene Activation System on Human Cytomegalovirus and Herpes Simplex Virus Type-1 Infections

Kwang-Hoon Choi, Ki-Ho Kim, and Hong-Jin Kim

College of Pharmacy, Chung Ang University, Seoul, 156-756, Korea

(Received March 16, 2000)

The tetracycline-controlled transactivator (tTA)-mediated gene activation system was examined in virus infected cells to determine its role in the control of gene expression. In the presence of tTA, the gene expression from the *tetO*-modified minimal promoter was efficiently activated in the uninfected cells, whereas essentially no activation was observed from the only minimal promoter without the seven direct repeats of 42 bp *tetO* sequences. However, essentially no activation was observed when only the minimal promoter was used, without the seven direct repetitions of the 42 bp *tetO* sequences. On the other hand, in the infected cells, a substantial background of β-glucuronidase expression was detected in the absence of tTA, even though tTA stimulated the gene expression by ~7-fold. This background expression indicates that the sequences within or nearby *tetO* are involved in the background stimulation of the gene expression by HCMV and HSV-1. These results suggest that the application of the tTA-mediated gene activation system may not be extremely useful for studying the biological roles of HCMV and HSV genes in the viral replicative cycles, because of the basal activity of the gene expression.

Key words: tTA, HCMV, Gene expression, Tetracycline

INTRODUCTION

In order to control the gene expression in a eukaryotic system, a tetracycline repressor protein (TetR)-mediated repression and activation system was developed. TetR-mediated repression was first reported in a plant system (Gatz *et al.*, 1992; Gatz and Quail, 1988) and in a mammalian system (Kim *et al.*, 1995). In this system, *tet* operator sequences, approximately positioned in the promoter and binding of TetR to the operator sequences, repressed the expression of the linked reporter gene. However, the presence of tetracycline can prevent the repressor from binding to its operator sequence and thereby induce the expression of the reporter gene.

TetR-mediated activation was first reported in mammalian cells (Gossen and Bujard, 1992; Gossen *et al.*, 1995). In this system, tetracycline-controlled transactivator (tTA) was generated by the fusion of TetR and the C-terminal activation domain of the VP16 domain of herpes simplex virus type-1 (HSV-1). tTA binds to the multiple *tetO* sequences on the minimal promoter, and thereby induces the expression of the linked gene. In the absence of tetracycline, tTA activates the gene expression from a minimal promoter containing multiple *tetO* sequences located upstream from the promoter. However, the presence of tetracycline prevents tTA from binding to its operator sequences, thereby leading to low-level basal expression from the minimal promoter. Although the tTA-mediated gene activation system for inducing gene expression has greatly facilitated the study of the biological roles of animal genes (Massie *et al.*, 1998; Obeist *et al.*, 1999), plant genes (Roder *et al.*, 1994; Weinman *et al.*, 1994; Masgrau *et al.*, 1997) and gene therapy (Onge *et al.*, 1996; Yu *et al.*, 1996; A-Mohammadi *et al.*, 1998), the application of the tTA-mediated activation system in human cytomegalovirus (HCMV) and HSV-1 has not been reported to date.

In this study, we examined whether the tTA-mediated gene activation system could be applied to HCMV and HSV-1 replication cycles in order to control viral gene expression. The gene expression of the reporter gene, under the control of the minimal promoter containing the *tetO* sequences, was controlled by the presence of tTA. However, the minimal promoter was activated by HSV-1 as well as HCMV infection, even in the absence of tTA.

Correspondence to: Hong-Jin Kim, College of Pharmacy, Chung Ang University, 221 Heuksuck-Dong, Dongjak-Gu, Seoul, 156-756, Korea
E-mail: hongjink@cau.ac.kr

This activation of the minimal promoter induced basal activity in the reporter gene expression of the tTA-mediated gene activation system. Therefore, we concluded that the tTA-mediated gene activation system may not be extremely useful for studying the function of the viral genes in the HSV-1 and HCMV replication cycles.

MATERIALS AND METHODS

Cells and virus

Human foreskin fibroblast (HFF) cells were isolated in our laboratory and used below passage 20. U373-MG cells were obtained from the American Type Culture Collection. Both of the cell types were grown in Dulbecco's modified Eagle medium (DMEM; GIBCO) containing 10% fetal bovine serum (GIBCO) and 25 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acids). The HCMV strain AD169 and the HSV-1 strain F were obtained from the American Type Culture Collection, and were propagated according to standard protocols.

Reporter gene constructs

pBg10PA was used as the parental plasmid, and consisted of the promoterless β-glucurionidase gene and the HCMV poly A signal. p7tetminBg, as described by Kim *et al.* (1998), contains seven direct repeats of the 42 bp *tetO* sequences (Gossen and Bujard, 1992), the HCMV minimal promoter (Gossen and Bujard, 1992), β-glucuronidase, and the HSV-1 thymidine kinase poly A signal (Jones and Muzithras, 1991). To construct pminBg, p7tetminBg was excised with *Pst* 1 and *Asp*718 and religated. pminBg consisted of the HCMV minimal promoter, the β-glucuronidase gene, and the poly A signal.

Transient expression and β-glucurionidase assay

HFF and U373-MG cells were transfected in 60-mm-diameter plates while they were 70% to 80% confluent. Ten micrograms of supercoiled plasmid DNA was transfected per plate in 2 ml DMEM, containing 200 μg of DEAE-dextran (Mw of 500, 000; Pharmacia) per ml. After 4 h at 37°C, the cells were shocked with 20% dimethyl sulfoxide in 1× HEPES-buffered saline (pH 7.05) for 2 min. The cells were washed twice with phosphate-buffered saline (PBS), and growth medium was added. After incubation for 24 h, these plates were infected with HCMV strain AD169 or HSV-1 strain F, at a multiplicity of 4 PFU per cell. Cell extraction was performed 24 h post-infection, and the assay was done, as previously described (Jones *et al.*, 1991). Alternatively, U373-MG cells were transfected in 60-mm-diameter plates using the standard calcium phosphate method (Sambrook *et al.*, 1988). Ten micrograms of supercoiled plasmid DNA was transfected per plate. After 7 h at 37°C, the cells were shocked with 15% glycerol in 1× HEPES-buffered saline (pH 7.05) for 2 min. The cells were washed twice with phosphate-buffered saline (PBS), and growth medium was added. Cell extraction was performed 48 h post-transfection, and the assay was done, as described above. The total protein in the cell extracts was determined by the Bradford method as described in the kit (Bio-Rad).

RESULTS AND DISCUSSION

The effect of tTA on the gene expression from the HCMV minimal promoter containing the *tetO* sequences

In order to determine whether the tTA-mediated activation system could be established in HCMV, we evaluated the effect of tTA on the gene expression from the HCMV minimal promoter containing the *tetO* sequences. We constructed an indicator plasmid (p7tetminBg) which contained seven direct repeats of the 42 bp *tetO* sequences (Gossen and Bujard, 1992), the HCMV minimal promoter (Gossen and Bujard, 1992), β-glucuronidase, and the HSV-1 thymidine kinase poly A signal (Jones and Muzithras, 1991). p7tetminBg was cotransfected with either pUHD15-1 (Gossen and Bujard, 1992), which expressed a chimeric TetR-VP16 fusion protein (tTA), or pUC19 into HFF cells using the DEAE-dextran method (Kim *et al.*, 1995). The transient expression was analyzed. In the presence of tTA, the expression from the *tetO*-modified minimal promoter was activated ~22-fold in the uninfected cells. However, essentially no activation was observed from pBg10pA when it contained only the β-glucuronidase gene without the promoter (Table I). On the other hand, there was a substantial background β-glucuronidase expression in the infected cells in the absence of tTA, even though tTA stimulated the gene expression by ~7-fold (Table I). These results suggest that the HCMV virus may mediate the stimulation of the gene expression through seven direct repetitions of the 42 bp *tetO* sequences or through the minimal promoter.

Table I. tTA activation of expression from *tetO*-modified HCMV minimal promoter plasmid

	β-Glucuronidase activity in[a] :			
	Virus infecton		No infection	
Plasmid	pUC19	PUHD15-1	pUC19	PUHD15-1
p7tetminBg	120 ± 20	750 ± 100	1 ± 1	22 ± 3
pBg10pA	3 ± 2	4 ± 2	1 ± 1	1 ± 1

[a]β-Glucuronidase activity (picomoles of product per 0.1 μg of protein per min) was determined after cotransfection of the indicated plasmids in the HCMV-infected transient expression system. Duplicate plates of HFF cells were infected with HCMV at a multiplicity of infection of 3 PFU per cell. Cotransfection with pUC19 was done as a negative control. The results shown are the means ± standard deviations of three independent transfections.

The effect of HCMV infection on the gene expression from the HCMV minimal promoter containing the *tetO* sequences

In order to determine whether *tetO* sequences mediate the stimulation of the gene expression by HCMV infection in the absence of tTA, we analyzed the effect of the gene expression from the minimal promoter by removing the *tetO* sequences. A construct (pminBg) was made by deleting the seven direct repeats of the 42 bp *tetO* sequences from p7*tet*minBg. The resulting construct (pminBg) contained the HCMV minimal promoter, the β-glucuronidase gene, and the poly A signal. Either p7tetminBg or pminBg was transfected into HFF cells using the DEAE-dextran method. On HCMV infection, the gene activation of p7tetminBg was approximately 18 times higher than the gene activation of pminBg (Table II). However, neither p7tetminBg nor pminBg was activated in the uninfected cells. In order to verify the possibility that this stimulation of the gene expression by HCMV infection was not specific to HFF cells, we also transfected the above construct into U373 cells using DEAE-dextran method. On HCMV infection of U373 cells, the gene activation of p7tetminBg was approximately 19 times higher than the gene activation of pminBg. However, neither p7tetminBg nor pminBg was activated in the uninfected cells, although a slightly higher level of background expression was found in the U373 cells than in the HFF cells (Table II). The similar levels of gene activation from p7tetminBg in both the HFF and U373 cells indicated that the sequences within or nearby *tetO* were involved in the background stimulation by HCMV.

Table II. Transactivation of the minimal promoter containig *tetO* sequence by HCMV infection

Plasmid	β-Glucuronidase activity in[a]:			
	HFF cells		U373 cells	
	No infection	Virus infection	No infection	Virus infection
p7tetminBg	1 ± 1	90 ± 10	20 ± 10	380 ± 50
pminBg	1 ± 1	5 ± 2	30 ± 15	20 ± 10
pUC19	1 ± 1	5 ± 3	20 ± 10	10 ± 5

[a]β-Glucuronidase activity (picomoles of product per 0.1 μg of protein per min) was determined after transfection of the indicated plasmids in the HCMV-infected transient expression system. Duplicate plates of either U373-MG cells of HFF cells were infected with HCMV at a multiplicity of infection of 3 PFU per cell. pUC19 was used as a negative control. The results shown are the means ± standard deviations of three independent transfections.

Table III. Transactivation of the minimal promoter containig *tetO* sequence by HSV-1 infection

Plasmid	β-Glucuronidase activity in[a]:	
	No infection	Virus infection
p7tetminBg	30 ± 10	410 ± 30
pminBg	30 ± 2	20 ± 15
pUC19	20 ± 10	10 ± 2

[a]β-Glucuronidase activity (picomoles of product per 0.1 μg of protein per min) was determined after transfection of the indicated plasmids in the HSV-1 infected transient expression system. Duplicate plates of U373-MG cells were infected with HSV-1 at a multiplicity of infection of 3 PFU per cell. pUC19 was used as a negative control. The results shown are the means ± standard deviations of three independent transfections.

The effect of HSV-1 infection on the gene expression from the HCMV minimal promoter containing the *tetO* sequences

In order to determine if other herpes viruses can activate the gene expression from the minimal promoter containing the *tetO* sequences, we also examined HSV-1. p7tetminBg, pminBg, and mock were each transfected into U373 cells, which were then superinfected the next day with HSV-1. Similar levels of gene activation from p7tetminBg were found on HSV-1 infection as in HCMV infection (Table III). However, almost no gene activation was found from pminBg or the mock on HSV-1 infection. Therefore, we believe that the sequences within or nearby *tetO* are involved in the background stimulation of the gene expression caused by HCMV and HSV-1.

The effect of HCMV immediate early proteins on the activation of the HCMV minimal promoter containing the tetO sequences

Because the HCMV minimal promoter containing the *tetO* sequences was activated in 24 h postinfection by HCMV, we speculated that HCMV immediate early (IE) proteins do activate the minimal promoter. In order to determine whether immediate early proteins are related to the activation of the minimal promoter containing the *tetO* sequences, we cotransfected p7tetminBg into U373 cells with either pSVH (Stenberg *et al.*, 1990) expressing IE1 and IE2 or pUC19 (negative control). In this transient experiment, p7tetminBg was not activated by pSVH (Data not shown). Therefore, we found that the minimal promoter containing the *tetO* sequences was not activated by HCMV IE1 and IE2 proteins.

ACKNOWLEDGEMENTS

This work was supported by the Research Support Program of Chung Ang University.

REFERENCES

Mohammadi, S. and Hawkins, R.E. Efficient transgene regulation from a single tetracycline-controlled positive feedback regulatory system. *Gene Therapy*, 5, 76-84 (1998).
Fishman, G., Kaplan, M.L., and Buttrick, P.M. Tetracycline-

regulated cardiac gene expression *in vivo*. *J Clin Invest*, 93, 1864-1868 (1994).

Gossen, M.and Bujard, H. Tight control of gene expression in mammalian cells by tetracycline-responsive promoter. *Proc. Natl. Acad. Sci. USA.*, 89, 5547-5551 (1992).

Gossen, M., Freundlieb, S., Bender, G., Muller, G., Hillen, W., and Bujard, H. Transcriptional activation by tetracyclines in mammalian cells. *Science,* 268,1766-1769 (1995).

Jones, T. R.and Muzithras, V.P. Fine mapping of transcripts expressed from the US6 gene family of human cytomegalovirus AD169. *J. Virol.*, 65, 2024-2036 (1991).

Jones, T. R., Muzithras, V.P., and Gluzman, Y. Replacement mutagenesis of the human cytomegalovirus genome. *J. Virol.*, 65, 5860-5872 (1991).

Kim, H-J., Gatz, C., Hillen W., and Jones, T.R. Tetracycline repressor-regulated gene repression in recombinant human cytomegalovirus. . *J. Virol.*, 69, 2565-2573 (1995).

Kim, H-J. and Kim, K. H. Characterization of tTA and its functional domain in tetracycline repressor-mediated gene repression system. *Arch. Pharm. Res.,* 21, 320-325 (1998).

Masgrau, C. Inducible overexpression of oat arginine decarboxylase in transgenic tobacco plants. *Plant J*, 11, 465-473 (1997).

Massie, B., Couture, F., Lamoureux, L. Mosser, D. D., Guilbault, C., Jolicoeur, P., Belanger, F., and Langelier, Y. Inducible overexpression of a toxic protein by an adenovirus vector with a tetracycline-regulatable expression cassette. *J. Virol.*, 72, 2289-2296. (1998).

Obeist, C., Hartl, M., Weiskirchen,, R., and Bister K. Conditional cell transformation by doxycycline-controlled expression of the MC29 v-myc allele *Virology*, 253, 193-207 (1999).

Roder, F.T., Schmulling, T., and Gatz, C. Efficiency of the tetracycline-dependent gene expression system: complete suppression and efficient induction of the rolB phenotype in transgenic plants. *Mol Gen Genet*, 243, 32-38 (1994).

Sambrook, J., Fritisch, E. F., and Maniatis, T. *Molecular cloning: a laboratory manual*, 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1998).

Stenberg, RM., Fortney, J., Barlow, SW., Magrane BP., Nelson JA., and Ghazal, P. Promoter-specific trans activation and repression by human cytomegalovirus immediate-early proteins involves common and unique protein domains. *J. Virol.*, 64, 1556-1565 (1990) .

St-Onge, L., Furth, PA. and Gruss, P. Temporal control of the Cre recombinase in transgenic mice by a tetracycline responsive promoter. *Nucleic Acids Res.*, 24, 3875-3877 (1996).

Weinmann, P., Gossen, M., Hillen W., Bujard H., and Gatz, C., A chimeric transactivator allows tetracycline-responsive gene expression in whole plants. *Plant J.*, 5, 559-56 (1994).

YU, Z., Redfern, C.S. and Fishman, G.I. Conditional transgene expression in the heart. *Circ Res*, 79, 691-697 (1996).