Arch. Pharm. Res. Vol. 16, No. 2, pp. 155-157, 1993

Note

DNA Transfection in SK-N-BE(2)C Human Neuroblastoma Cells

Myung Koo Lee

College of Pharmacy, Chungbuk National University, Cheongju 360-763, Korea

(Received December 29, 1992)

DNA transfection conditions were investigated by calcium phosphate-DNA co-precipitation in SK-N-BE(2)C human neuroblastoma cells. The DNA plasmid of TH2400CAT was used in which rat tyrosine hydroxylase gene was inserted into chloramphenicol acetyltransferase reporter gene. The transfection efficiency was 25~30% and the method was simple and reproducible. So, the method will be a good tool for transient transfection analysis.

Key words: Transfection analysis, Human neuroblastoma cell, Tyrosine hydroxylase, Chloramphenicol acetyltransferase assay

INTRODUCTION

DNA transfection in mammalian cells is a covenient method for expressing mature proteins from cloned mammalian cDNA. The most commonly used methods are transfections with DEAE-dextran (Sussman and Milman, 1984), with calcium-phosphate (Chen and Okayama, 1987), with cationic liposome (Felgner *et al.*, 1987; Hatzfeld and Hatzfeld, 1991) and by electroporation (Anderson and Evans, 1998). Serum-supplemented media are used after transfection to allow growth of transfected cell, replication of transfected plasmids and expression of inserted cDNA. Serum-free defined media are often used for increasing the transfection efficiency (Hatzfeld and Hatzfeld, 1991).

The SK-N-BE(2)C cells, derived from a human neuroblastoma, express the catecholamine biosynthetic enzymes such as tyrosine hydroxylase (TH), aromatic L-amino acid decarboxylase and dopamine β-hydroxylase (Kim *et al.*, 1993). Therefore, the SK-N-BE(2)C cells have been used as a model system for the gene regulation using transient transfection analysis.

The rat TH gene has potential cis-acting motifs such as AP1, AP3, POU/Oct, SP1 and c-AMP-response element within −2400 bp (Coker *et al.*, 1988). The TH upstream region within −2400 bp was fused to the bacterial chloramphenicol acetyltransferase (CAT) gene as a reporter gene (TH2400CAT plasmid). The plasmid preparation of TH2400CAT has been described elsewhere (Kim *et al.*, 1993).

In this study, the optimal conditions of transfection in SK-N-BE(2)C cells using the TH2400CAT plamid are investigated by the calcium phosphate-DNA co-precipitation. The method of calcium phosphate-DNA co-precipitation in SK-N-BE(2)C cells reproducibly provides a good transfection efficiency.

Correspondene to: Myung Koo Lee, College of Pharmacy, Chungbuk National University, San 48, Kaeshin-Dong, Cheongju 360-763, Korea

MATERIALS AND METHODS

Cell culture

The human neuroblastoma cells SK-N-BE(2)C were routinely grown in 60 mm tissue culture-dish in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum (FCS) and penicillin (100 units/ml)/streptomycin (100 μg/ml). The confluent cells were harvested by trypsinization with 1 ml of phosphate-buffered saline (PBS) containing 0.05% trypsin and 0.02% EDTA. Trypsinized cells were added in DMEM-FCS medium and incubated at 37℃ in 5% CO_2 humidified atmosphere until transfection.

DNA plasmids

TH2400CAT plamid was prepared as previously described (Kim *et al.*, 1993). This plasmid was amplified in MC1061 *E. coli* strain, extracted by the alkaline procedure and purified two times on a CsCl gradient. pBL-CAT plasmid without any inserted cDNA was used for control. For transfection analysis, all plasmid DNA were purified twice by phenol-chloroform extraction and ethanol precipitation in the presence of 2.5 M ammonium acetate.

In all experiments, pRSV βGal plasmid containing the β-galactosidase gene linked to the RSV promotor/enhancer was included as an internal control for the different transfection efficiencies between experi-

ments.

DNA transfection with calcium phosphate and CAT assay

Transfection of reporter plasmids into SK-N-BE(2)C cells was performed by the calcium phosphate-DNA co-precipitation method (Chen and Okayama, 1987). The cells, which reached approximately 40% confluency (ca. 2×10^5 cells/cm^2) in 60 mm Falcon tissue culture dish, received 4 μg of TH2400CAT plasmid and 1 μg of pRSV βGal plasmide by Ca^{2+}-DNA co-precipitation (total DNA amount 5 μg, total volume 500 μl in 2× HEPES buffered solution 25 mM, pH 7.05 with 0.125 M Ca^{2+}). The cells were exposed to the precipitate for 12 hrs and then medium was replaced with DMEM-FCS. After a further 24 hr incubation, the cells were washed with PBS (2 ml) and harvested by scraping with a rubber policeman in PBS. Extracts were prepared by resuspending cells in 200 μl of 0.25 M Tris-HCl buffer (pH 8.0) followed 3 times of freeze-thaw cycles. The extracts were heated at 60℃ for 10 min to inactivate endogenous acetylase.

CAT activity was measured as described (Lewis *et al.*, 1987) using 0.5 μCi [^{14}C] chloramphenicol, 0.25 mM n-butyryl Co-A as a cofactor and 20 μl of cell extracts in 100 μl of 0.25 M Tris-HCl buffer (pH 7.5) at 37℃ for 30 min. Butyrylated reaction products were extracted with the mixture of Pristane-xylene (2 : 1) and the extracts were counted by the scintillation counter (Beckman, USA). Accurate amount of cell extracts were adjusted by normalizing each transfection efficiency by β-galactosidase assay (Arvidson *et al.*, 1991; Kim *et al.*, 1993). In most experiments, the variation of β-galactosidase activity was within the range of 15%.

Forskolin and isobutyl methylxanthine were added directly to the media 12 hrs prior to harvest. To check the efficiency of transfection, small aliquots of the harvested cells were tested for the expression of β-galactosidase by the histochemistry using the x-gal solution (Kim *et al.*, 1993).

RESULTS AND DISCUSSION

Human neuroblastoma cells, presumably of neural crest origin, have been utilized as *in vitro* models of neural differentiation. One of the human neuroblastoma cells, SK-N-BE(2)C, could reproducibly be transfected with high frequency.

The quantity of DNA required for an optimal signal in transient transfections varies with cell types. The DNA amount using TH2400CAT and pRSV βGal plasmids were investigated by the calcium phosphate-DNA co-precipitation. The DNA amount of TH2400CAT from 1 μg to 15 μg increased the CAT activity: 4 μg of TH2400CAT and 1 μg of pRSV γGal plasmids were selected (total DNA amount, 5 μg).

The stable CAT activities were obtained by the exposure time of 3-16 hrs after transfection. Longer period of exposure to the precipitate altered the cell viability. In the experiment, the exposure time of 12 hrs was selected and then media were replaced with fresh DMEM-FCS.

The SK-N-BE(2)C cells, which reached the confluency of 40% (ca. 2×10^5 cells/cm^2), were used for stable transfection. The cell confluency lower than 30% showed no good transfection efficiency (less than 15%). At higher confluency than 50%, cells were overgrown during exposure periods.

PC12 cells, rat pheochromocytoma have been reported to be treated with glycerol or DMSO to increase transfection efficiency (Lewis *et al.*, 1987; Kilboure *et al.*, 1991). And the poly-L-lysine-, dextran-, collagen- or gelatin-treated culture dish was usually adopted prior to the transfection procedure because cells, when handled, were easily floated from the culture dish (Kilboure *et al.*, 1991). However, glycerol or DMSO treatment in SK-N-BE(2)C cells didn't increase the CAT activity. SK-N-BE(2)C cells strongly attached to the surface of plates without a special treatment such as poly-L-lysine or gelatin. The optimal conditions of transfection in the SK-N-BE(2)C cells were summarized in Table I.

PC12 cells showed a very low transfection efficiency using a rough-size of calcium phosphate-DNA precipitate because the cell viability was altered by the rough particles and by the longer exposure periods (Kim *et al.*, 1993). In SK-N-BE(2)C cells, the particle size of precipitate had little effect on the transfection analysis. But fine and consistent particle sizes were required for a stable and satisfactory transfection (data not shown).

In this method, serum-containing medium DMEM-FCS was used and a newborn calf serum could be used instead of FCS. Serum-free conditions were not good for cell viability and expression (unpublished observation). But serum-free media in COS cells showed a good transfection efficiency by DEAE-dextran/chloro-

Table I. Comparison of the transfection conditions between SK-N-BE(2)C and PC12 cells

Cell lines	SK-N-BE(2)C	PC12[a]
Total DNA amount[b]	5 μg	10 μg
Transfection efficiency	25-30%	2-5%
Cell confluency (60 mm-culture dish)	40%	50%
Exposure time after transfection	12 hr	6 hr
glycerol treatment	non	+

[a]The transfection conditions for PC12 cells were cited form Kim *et al.* (1993).

[b]HEPES buffer (25 mM, pH 7.05) with Ca^{2+} (0.125 M) was used for calcium phosphate-DNA co-precipitation.

Table II. CAT activities obtained from the TH2400CAT plasmid using SK-N-BE(2)C cell extracts

	CPM	Conversion rate, %
pBLCAT	3020	1.5
TH2400CAT	40450	20.7
TH2400CAT + Forskolin	82460	42.3

The cell extracts of 20 μl obtained from control plasmid pBLCAT and TH2400CAT plasmid were used for CAT assay. Forskolin 10 μM was treated for 12 hrs after transfection and then cells were incubated for 12 hrs. The conversion rate was the percentage of butyrylated products vs. substrate ^{14}C-chloramphenicol. All experiments were performed in triplicate.

quine (Hatzfeld and Hatzfeld, 1991).

The transfection efficiency of 25-30% in SK-N-BE(2)C cells were shown by the x-gal solution method (n=20), whereas that of 2-5% in PC12 cells were shown (Kim *et al.*, 1993). The CAT activities obtained from the TH 2400CAT plasmid and the control pBLCAT Plasmid were shown in Table II. The TH2400CAT activity was increased by the treatment of forskolin, which is an activator of adenylate cyclase (Lewis *et al.*, 1987) (Table II). The method was well applied to study the mechanism of TH gene regulation (Kim *et al.*, 1993).

The transfection conditions are simple and reproducible in using the popular technique, calcium phosphate-DNA co-precipitation. Therefore, the method will be a good tool for transient transfection analysis.

REFERENCES CITED

Anderson, G. L. and Evans, G. A., Introduction and expression of DNA molecules in eukaryotic cells by electroporation. *Bio Techniques,* 6, 650-655 (1988).

Arvidson, D. N., Youderian, P., Schneider, T. D. and Stormo, G. D., Automated kinetic assay of β-galactosidase activity. *Bio Techniques,* 11, 733-738 (1991).

Chen, C. and Okayama, H., High efficiency transformation of mammalian cells by plasmid DNA. *Mol. Cell. Biol.,* 7, 2745-2752 (1987).

Coker, G. T., Vinnedge, L. and O'Malley, K. L., Characterization of rat and human tyrosine hydroxylase gene: Functional expression of both promotors in neural and non-neural cell types. *Biochem. Biophys. Res. Comm.,* 157, 1341-1347 (1988).

Felgner, P. L., Gadek, T. R., Holm, H., Roman, R., Chan, H. W., Wenz, M., Northrop, J. P., Ringold, G. M. and Danielsen, M., Lipofectin: A highly efficient, lipidmediated DNA-transfection procedure. *Proc. Natl. Acad. Sci. USA,* 84, 7413-7417 (1987).

Hatzfeld, J. and Hatzfeld, A., DNA transfection in COS cells: A low-cost serum-free method compared to lipofectin. *Bio Techniques,* 11, 313-318 (1991).

Kilbourne, E. J., McMahon, A. and Sabban, E. L., Membrane depolarization by isotonic or hypertonic KCl; Differential effects on mRNA levels of tyrosine hydroxylase and dopamine β-hydroxylase mRNA in PC12 cells. *J. Neurosc. Methods,* 40, 193-202 (1991).

Kim, K. S., Lee, M. K., Carroll, J. and Joh, T. H., Both the basal and inducible transcription of tyrosine hydroxylase gene are dependent upon a c-AMP-response element. *J. Biol. Chem.,* (1993), in press.

Lewis, E. J., Harrington, C. A. and Chikaraishi, D. M., Transcriptional regulation of the tyrosine hydroxylase gene by glucocorticoid and cyclic AMP. *Proc. Natl. Acad. Sci. USA,* 84, 3550-3554 (1987).

Sussman, D. J. and Milman, G., Short-term, high-efficiency expression of transfected DNA. *Mol. Cell. Biol.,* 4, 1641-1647 (1984).