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NOVOBIOCIN INHIBITS THE SV40 ENHANCER ACTIVITY

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Using a transient expression assay, we have analysed the effect of novobiocin, DNA topoisomeraseII inhibitor, on simian virus 40(SV40) enhancer activities. We used the recombinant clones containing typeI or II collagen promoters placed upstream of the bacterial chloramphenicol acetyltransferase (CAT) gene with or without SV40 enhancer.

We observed the expected increase in CAT activities due to the presence of the SV40 enhancer. Interestingly, CAT gene expression of the enhancer-containing constructs were inhibited more sensitively by novobiocin than that of the enhancer-less construct. This findings lead us propose that DNA superhelicity mediated by topoisomeraseII is one of the important factor for the manifestation of SV40 enhancer activity.

Transcriptional enhancer sequences in both cellular and viral genomes have been shown to play an important role in the regulation of gene expression (1). As an enhancer acts in both distance and orientation independent manner, one can speculate that an enhancer may function separately from the transcriptional machinery. It has been shown that limiting amounts of cellular factor bind to enhancer regions (2) and that SV40 enhancer induces an altered chromatin structure (3). It has also become evident that DNA topoisomerase II induces DNA supercoiling and an active conformation of chromatin (4) and that this is required for efficient expression both in vivo and in vitro (4,5).

The topoisomerase II cleavage sites have been mapped at nuclease hypersensitive sites in the SV40 minichromosome (6).

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Further, Villeponteau et al. have recently found that an actively transcribed chicken β-globin gene in chromatin is sensitive to cleavage by DNase I and that this sensitivity is lost after the treatment of novobiocin.
Topoisomerase II is required to maintain open DNase-sensitive states of chromatin (7).

To study whether DNA supercoiling, mediated by DNA topoisomerase II, is involved in the transcriptional activation of SV40 enhancer, we used transient CAT expression assays and examined the effect of novobiocin, a specific inhibitor for DNA topoisomerase II, on CAT expression directed by two different promoter-CAT constructs with and without the SV40 enhancer.

MATERIALS AND METHODS

Enzymes and Reagents : All enzymes for plasmid construction were purchased either from New England Biolabs or Bethesda Research Laboratory. Novobiocin was obtained from Sigma Chemicals. Acetyl-CoA was from PL-Pharmacia. Silica gel plate 1B2 was from J.T.Baker Chemical Company. [14C]-chloramphenicol was from New England Nuclear.

DNA Transfection and CAT Assay : Approximately 2×10^5 cells were plated on to 35mm dishes 24 hours before the addition of precipitate. The precipitate was applied to the cells and incubated at 37°C. Four hours later, the cells were washed with serum free medium 3 times and refed fresh medium. Usually NIH3T3 cells were transfected with 10ug plasmid DNA per 35mm dish by the calcium phosphate precipitation method. The CAT enzyme activity was analyzed 48 hours after transfection in the presence of various concentrations of novobiocin as described previously (8,9).

RESULTS

The recombinant clones used in the present study have been constructed as shown in Figure 1. We used the two different promoters from mouse α2(I) collagen gene and rat α1(II) collagen gene. These genes are expressed in fibroblast and chondrocyte respectively. However, type II collagen promoter (pRcolIIHS) could express CAT activity weekly in both fibroblast and chrondrocyte and mouse α1 type I collagen promoter (paZ1003) functions in fibroblast but not in chondrocyte (data not shown). So, we used NIH3T3 cells to examine the effect of novobiocin on CAT expression by transfection of these recombinants. These promoters were rather weak and CAT expression was increased 10 to 50 fold in a typical experiment by the presence of SV40 enhancers

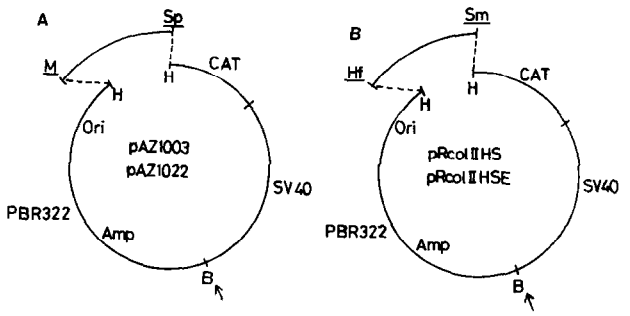


Figure 1 Schematic representation of the recombinant clones used in the present study.

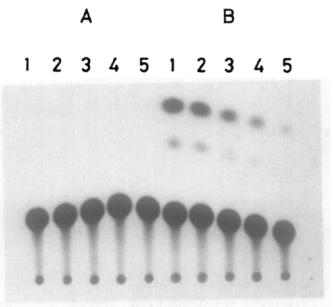
(A) pAZ1003, pAZ1022 (SV40 enhancer +) : These plasmids are kindly provided by Dr. A. Schmidt (National Cancer Institute)(10) 2kb of the MstII/SphI fragment of the mouse α(I) collagen gene promoter region was inserted by means of Hind III linkers into the Hind III site of the pSVO-CAT. The SV40 enhancer contains SV40 sequences between +113 and +270 in the SV40 map.

(B) pRcolIIHS, pRcolIIHSE (SV40 enhancer +) : 450bp of HinfI/SmaI fragment of the rat type (II) collagen gene promoter region was inserted in the HindIII site of pAZ1003 and pAZ1022 instead of the mouse genomic fragment. (11)

The locations of relevant restriction sites are shown : M=MstII ; Sp=SphI ; H=HindIII ; B=BamHI ; Hf=HinfI ; S=SmaI. Sites underlined were destroyed during construction.
Arrow indicates the location of SV40 enhancer.

(data not shown). The exact magnitude of enhancer effects varied in separate experiments. Second, we evaluated the effect of novobiocin on the viability of NIH3T3 cells. The cells viability 48 hours after the addition of novobiocin (400 μg/ml) inhibited cell replication strongly, but the cells can divide in less than 200 μg/ml of novobiocin (data not shown). So, we decided to use relatively low concentrations of novobiocin 0 to 200 μg/ml.

Figure 2 shows the effect of novobiocin on CAT expression from the type II collagen promoter-CAT construct with and without enhancer. CAT expression of pRcolIIHS is low but increased about 45 fold in the resence of the SV40 enhancer (figure 2 compared with lane 1 in (A) and (B)). The CAT expression of pRcolIIHS seems to decrease gradually by the increasing levels of novobiocin. However, CAT expression of pRcolIIHSE is rapidly reduced by increasing levels of novobiocin.



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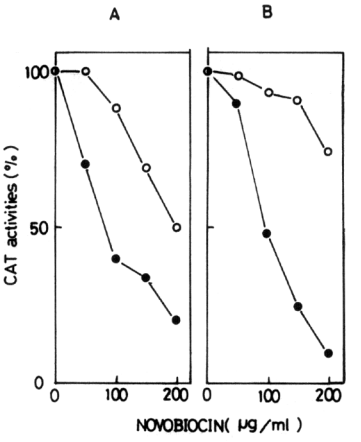

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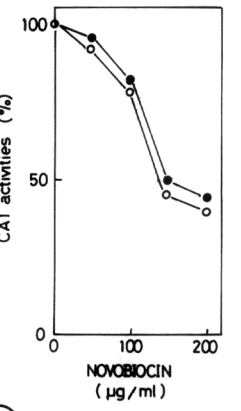

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Figure 2 Effect of novobiocin on CAT expression in NIH3T3 cells transfected with pRcolIIHS and pRcolIIHSE.

pRcolIIHS (A) and pRcolIIHSE (B) were transfected into NIH3T3 cells as described in the Methods. After transfection the cells were incubated for 48 hours in the presence of various concentrations of novobiocin, as follows : lane 1: 0, lane 2:50, lane 3:100, lane 4:150, lane 5: 200 μg/ml. CAT assays and thin layer chromotography were performed as described.

Figure 3 Effect of novobiocin on CAT expression directed by two different promoter-CAT constructs with and without SV40 enhancer. The levels of CAT activity were plotted as the percent of activities obtained in the absence of novobiocin.

(A) pAZ1003 (open circle), pAZ1022 (closed circle)

(B) pRcolIIHS (open circle), pRcolIIHSE (closed circle)

Transfection experiments were done several times. For each experiment, quantitation was achieved by the count of acetylated 14C-chloramphenicol on TLC plates.

Figure 4 Effect of novobiocin on SV40 enhancer activity. Relative enhancement of CAT expression by the SV40 enhancer element was expressed as the percent of enhancement obtained in the absence of novobiocin.

pAZ1003 and pAZ1022 (open circle)

pRcolIIHS and pRcolIIHSE (closed circle)

Figure 3 shows the effect of novobiocin on CAT expression by two different promoter-CAT constructs with and without the SV40 enhancer. CAT expression is expressed as a percent of maximum activities obtained in the absence of novobiocin. These results clearly show that CAT expression of the enhancer-containing constructs, pAZ1022 and pRcolIIHSE, were inhibited more by

novobiocin than that of enhancer-less construct. Figure 4 also shows that the relative enhancement of CAT expression by the SV40 enhancer expresses as a percent of maximum enhancement at various levels of novobiocin. The data in figure 4 indicate again that enhancer activities are clearly repressed by novobiocin.

Since the number of cells was 100% at 100 μg/ml, and 90% at 200 μg/ml as compared with the cell number in the absence of novobiocin, this inhibition of CAT expression by novobiocin did not seem to be related to reduced cell number (data not shown).

DISCUSSION

We have found that SV40 enhancer activities are repressed by the DNA topoisomerase II inhibitor, novobiocin. This suggests that DNA superhelicity is one of the important factors for the manifestation of SV40 enhancer activities. It has been shown that both generation of an altered chromatin structure and the binding of cellular factors could be a part of the molecular action of enhancer elements (2,3) and that DNA topoisomerase II be responsible for maintaining the dynamic chromatin structure required for efficient transcription (4,5,14). Recently it has been also shown that novobiocin blocks the transcription by inhibiting formation of preinitiation complex at ATP-dependent step (15), or by inhibiting specific protein-DNA interaction (16). Thus, we can speculate that DNA topoisomerase II may be one of the transacting cellular factors affecting the gene expression. SV40 enhancer fully functions in vivo compared with in vitro transcription systems by using linearized DNA template. The competition assay shows enhancer elements interact with the limited amount of trans-acting factors in a cell or tissue specific manner.(3) But topoisomerase II is a prominent component of the nuclear scaffold or matrix (14). So rather than assuming that DNA topoisomerase II may interact with the enhancer region, we have considered the possibility that DNA superhelicity may change the binding affinity of the trans-acting factor to enhancer elements.

It has been shown that topoisomerase II may be present at specific sequences in chromosome loops of the nuclear scaffold or matrix (14,17). In the case of SV40 minichromosone, the specific topoisomerase II cleavage site is located at nucleotide 270 of the SV40 map near the enhancer (6). Therefore, DNA topoisomerase

II may act alone or together with other trans-acting factors in a sequence specific manner. It has been shown that DNase I hypersensitive sites can be induced in cis by SV40 sequences (18). They speculate that SV40 early region is the binding site of a trans-acting factor(s) which direct(s) the binding of a DNA gyrase-like activity that induces DNA supercoiling. At present, the function of enhancer binding factor is still obscure. However, the alteration of the DNA topology by DNA topoisomerase II might regulate gene expression, in part, by changing the binding affinity of trans-acting regulatory proteins. It is of interest to examine whether or not novobiocin specifically inhibits the gene activation of other classes of enhancer elements such as retrovirus and immunoglobulin gene enhancer (1), and examine the effect of another topoisomeraseII inhibitors such as m-AMSA, VP-16 and VM-26 on the SV40 enhancer activity.

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