Differential influence of DNA supercoiling on in vivo strength of promoters varying in structure and organisation in *E. coli*

G. Jyothirmai, Rakesh K. Mishra*

Centre for Cellular and Molecular Biology, Hyderabad 500 007, India

Received 18 January 1994

Abstract

DNA supercoiling is known to influence promoter activity in vitro and in vivo in a promoter-dependent manner in prokaryotes. In order to investigate how topology may influence promoter function, we have studied two kinds of promoter variants, (i) where only the spacer region is altered, and (ii) where the same promoter is tandemly repeated in either the same or opposite orientation. These promoters respond very differently to alterations in DNA supercoiling, suggesting that the overall structure of the promoter and its context contribute to the differential response to alterations in supercoiling in vivo.

Key words: Transcription; Promoter; DNA supercoiling; *Escherichia coli*

1. Introduction

The topological state of DNA and its transcriptional activity are intimately related. While transcription initiation is influenced by alteration in DNA supercoiling, elongation of transcripts causes alteration in the topological state of DNA, leading to the accumulation of positive supercoiling ahead and negative supercoiling behind the transcribing RNA polymerase [1]. It is likely that the topological state and transcriptional activity of DNA could influence each other and provide a means of gene regulation. In prokaryotes, such a mode of gene regulation has been observed [2] which suggests that regulatory mechanisms transduce signals for the regulation of such genes by altering the topological state of regulatory sequences. It would also suggest that different regulatory sequences (including promoters) respond differently to the supercoiling-mediated signal, and that the response is sensitive, with a small change in supercoiling leading to a variety of changes in the transcriptional activity of different promoters (reviewed in 3,4). In prokaryotes, the superhelical density (σ) of DNA is maintained by a homeostatic mechanism using the mutually antagonistic action of topoisomerases [5]. A signalling system can act either directly by perturbing this homeostasis in a sequence-specific manner or indirectly by changing the chromatin structure in the vicinity of the target gene [6] to alter the topological state and hence the transcriptional activity.

Several in vivo studies suggest that changes in supercoiling can have different effects on the transcriptional efficiency of promoters [7,8]. In vitro studies have shown that supercoiling has a profound influence on transcriptional efficiency of many promoters [9,10], although no supercoiling response element/module could be identified, leading to a general belief that it is the overall promoter structure that mediates this influence of supercoiling [9]. However, no satisfactory explanation is available to account for the varied response of promoters to the change in supercoiling of DNA.

We have studied two sets of promoters. In one set the prmup-1 promoter spacer region sequence is varied whilst the spacer length is kept constant (Fig. 1). In the other set, different repeats of the T7A0 promoter are combined in different orientations (Table 1). The in vivo transcription activity at natural or reduced superhelical density was measured by assaying for the product of a reporter gene driven by these promoter constructs. This gives an insight into how transcriptional activity of such promoter variants respond to alterations in DNA supercoiling.

2. Materials and methods

2.1. Promoter constructs

Plasmids containing prmup-1, s(wt), and its variants, s(CT), s(TT), s(AT), s(CC), s(GG) and s(CG), were obtained from Dr. Peter deHaseth [11]. In these plasmids the promoters drive the transcription

*Corresponding author.

Present address: Laboratoire de Biophysique Moleculaire, Universite de Bordeaux II, 146, Rue Leo-Saignat, 33076 Bordeaux Cedex, France. Fax: (33) 57 57 10 15.

of full β-galactosidase coding DNA. Plasmids pJ1, pJ2, pJ5, pJ6 and pJ7 were constructed as follows: pAR1350 containing the T7A0 promoter was digested with *Bam*HI to excise the 262 bp promoter containing fragment, Fig. 2A. This was ligated to *Bam*HI-linearized pMU575, a single copy plasmid containing a promoterless β-galactosidase gene with a multiple cloning site upstream, shown in Fig. 2B, and used to tranform lac⁻ CSH26 *E. coli* cells. Using MacConky lactose agar or X-gal agar plates, β-galactosidase-positive clones of different colour intensity were picked and analysed further. In all the cases *Bam*HI digestion yielded the 262 bp fragment containing the promoter. The size of the insert in these plasmids was estimated by *Sal*I and *Hind*III double digests. The orientation of the promoter containing fragments, with respect to the β-galactosidase coding sequence, was determined by the following restriction digestion analysis. (i) Plasmids were digested with *Sal*I, end labelled and digested again with *Pst*I. A series of partial or complete digestions were carried out with this DNA using either *Fok*I or *Kpn*I. (ii) Similar analysis was performed with the DNA first digested with *Hind*III, radiolabelled and then digested with *Xba*I. By estimating the size of various fragments thus generated, the orientation of each promoter insert was determined (Table 1).

2.2 Promoter activity assay

β-Galactosidase activity was measured by using the published procedure [12]. The effect of reduced supercoiling was analysed by adding the gyrase inhibitor, novobiocin (300 μg/ml) to cultures at the start of growth. The averaged results of ten individual assays are shown in the figures. All the promoters were assayed simultaniously each time to yield consistant data regarding relative activities. The analysis of the topoprofile of a 3 kb plasmid on a chloroquine agarose gel showed a shift of the topoprofile by 3 superhelical turns in the plasmids isolated from novobiocin-treated cells. This indicated a decrease of 0.01 in the negative superhelical density (σ). Considering the natural σ to be 0.05, the treatment with novobiocin caused a 20% decrease in supercoiling.

3. Results and discussion

Since DNA undergoes various conformational changes during the multistep process of transcription initiation (13–15), it is likely that, in addition to the conserved regions (−10 and −35 boxes), the conformational flexibility is an essential component of the DNA sequence that constitutes a promoter [16,17]. Such a flexibility is also subject to modulation by DNA supercoiling (18–20). The promoters studied here address these aspects of 'promoter instruction' in *E. coli*. We discuss the results obtained in the context of this multi-tier dynamic nature of promoter function.

Fig. 1 shows the activity of s(wt) and its variants, where only an unconserved 9 bp sequence has been altered. This alteration leads to profound differences in the in vitro and in vivo promoter properties. In the presence of novobiocin, the decrease in the in vivo promoter activ-

Table 1
Properties of T7A0 promoter constructs

Promoter	Size	Orientation	β-Gal activity (Miller units)	
			− novobiocin	+ novobiocin
pJ2	Single		4,400	2,600
pJ5	Dimer		25	26
pJ6	Dimer		117	80
pJ1	Trimer		48	46
pJ7	Trimer		122	85

Size refers to the number of repeats of the T7A0 promoter inserted in the *Bam*H1 site of pMU575 (see Fig. 2A). The orientation angle on top of the line (representing promoter) points toward the polymerase movement.

ity is minimal (less than 5%) for s(wt) and s(TT), very small (10–15%) for s(CC) and s(GG) and moderate (30%), in the case of s(TA), s(CG) and s(CT) (Fig. 3). The comparision of promoter strength and supercoil sensitvity reveals that topology contributes to transcription from a promoter independently of its inherent strength.

It has been shown before that non-conserved regions can also influence the properties of a promoter in vivo. The sequence alterations studied here have the potential to undergo micro-conformational changes or even adopt non-B DNA conformations under superhelical tension. The differential response to changes in supercoiling may be attributed to the influence of such structural elements on promoter activity [21,22]. The results suggest that the greater strength of s(CG), s(CT) and s(TA) promoters is, at least in part, contributed by negative supercoiling. Here, one third of the promoter strength comes from secondary and tertiary structures which exist only at physiological superhelical density and not when σ is lowered. In contrast to the proposition that weaker promoters may respond more to supercoiling in vitro [9], we observe that promoter strength does not influence the ability of a promoter to respond to alterations in supercoiling in vivo.

T7A0, which is known to be a very strong promoter, gave β-galactosidase activity of 4,400 Miller units. All the constructs other than the monomer result in significantly lower activity. These combinations may have been preferentially selected over constitutively expressed very
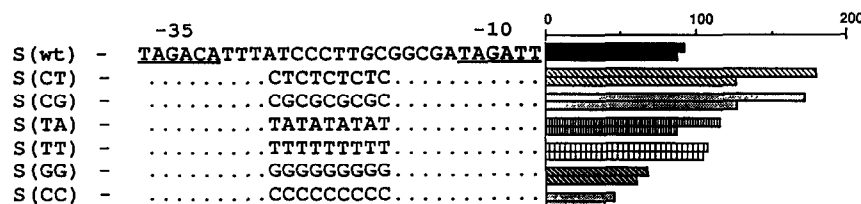
```
                  -35                        -10     0         100         200
S(wt)  -  TAGACATTTATCCCTTGCGGCGATAGATT   |____|____|____|____|
S(CT)  -  ........CTCTCTCTC...........
S(CG)  -  ........CGCGCGCGC...........
S(TA)  -  ........TATATATAT...........
S(TT)  -  ........TTTTTTTTT...........
S(GG)  -  ........GGGGGGGGG...........
S(CC)  -  ........CCCCCCCCC...........
```

Fig. 1. Sequence of prmup-1 promoter variants and their relative strength in vivo. The conserved hexanucleotide regions, −10 and −35 boxes, are underlined. The bar diagram shows the β-gal activity (in Miller units) driven by these promoters in the absence (upper bar) or presence of novobiocin (lower bar).
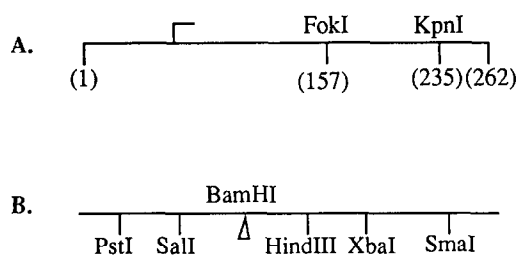
Fig. 2. (A) Restriction sites of T7A0 promoter fragment (the 262 bp BamHI fragment excised from pAR1350). Only unique sites are marked. The angle on top indicates tha direction of transcription from this promoter. (B) Restriction map of pMU575 flanking the BamHI site used for cloning the promoter. Only unique sites are marked. The β-gal coding region is downstream of the SmaI site.

strong constructs which might be lethal or cause poor growth. The strongest promoter known, T7A1, when used in similar studies did not yield any construct with activity greater than 100 Miller units, suggesting that constructs with very strong promoters are lethal and that only mutated variants with lower strength are isolated. The results summarised in Table 1 also reveal that these different combinations produce a varied response to reduction in supercoiling caused by novobiocin treatment, in addition to different in vivo activity.

As the movement of RNA polymerase leads to accumulation of positive supercoiling in front and negative supercoiling behind its point of association with DNA, such a twin domain becomes extremely important in determining promoter escape from initiation to elongation mode (for productive transcription) in a context where promoters are clustered together [23]. There are two ways by which clustered promoters influence each other: (i) when two promoters are very close, binding of RNA polymerase to one may sterically hinder binding to the other, or (ii) binding of one enzyme molecule at a promoter may withhold the promoter escape of a second enyme molecule at the neighbouring promoter by topological means. The very low activity of pJ5 can be explained by simultaneous occupation of the two promoters by RNA polymerase leading to fixation in the three dimensional structure which is poorly resolved (to let the polymerase track down) at the superhelical densities in these experiments. A third polymerase molecule in the opposite orientation, in pJ1, seems to release this stalemate to some extent, either by preventing occupation of the middle promoter or by generating locally very high negative supercoiling. In the case of pJ7, which is the inverse arrangement of the pJ1 combination, it is clearly seen that the polymerase transcribing from the

proximal promoter does escape. This may also explain in part the difference in activity observed in pJ1 and pJ5. The dimer combination of pJ6 is supercoil-sensitive in this range, like pJ7, although the actual mechanisms underlying this similarity are likely to be different. In the case of pJ6 the opposing polymerases may be able to 'bulldoze' through the other complex or pre-empt the formation of that complex assisted by the natural level of supercoiling. Lowering supercoiling decreases the efficiency of this process. The high strength of pJ2 and its sensitivity to change in supercoiling is expected as T7A0 is known to be a strong promoter and initiate transcription more efficiently at supercoiled templates [15].

These results demonstrate that supercoiling and promoter activity are linked in a promoter specific manner and in a cotextual sense. This may also suggest that when taken out of context, away from their normal chromosomal position, regulatory sequences may not retain original properties. The primary sequence of a promoter DNA, by virtue of confering unique structural and dynamic features, determines the kinetics and thermodynamics of various steps of transcription initiation that are unique to the promoter [14,15]. Alteration in supercoiling would bring about global change in these structural determinants of promoter properties and functionally modulate the promoter. Screening for promoter mutants using altered supercoil generating media (containing inhibitors of topoisomerases, for example) may give further insight into the molecular aspects of promoter strength and supercoil sensitivity.

DNA is associated with basic histone-like proteins in the form of a highly organised chromatin in prokaryotes [6,24]. A change in topological state of DNA would result in conformational changes to the chromatin, which then translate this change to a decrease or increase in the transcriptional activity of the promoter. The features of a promoter leading to such a response are not clear at the moment. Chromatin structure around these promoters might lead to differential organisation of the chromatin at these sequences, which may in turn govern the strength and supercoil sensitvity. Further, it can be envisaged that between two proximally situated promoters a topologically isolated niche is created [7,19], analogous to the typical chromatin domain of eukaryotes [25], which can influence expression of genes by topological means.

Fig. 3. Comparison of the effect of DNA supercoiling on prmup-1 and its variants. Promoters are grouped according to their strength (β-gal activity in Miller units) and response to decreases in supercoiling (reduction in promoter strength by novobiocin treatment).

References

[1] Liu, L.F. and Wang, J.C. (1987) Proc. Natl. Acad. Sci. USA 7024–7027.

[2] Higgins, C.F., Dorman, C.J., Stirling, D.A., Waddell, L., Booth, I.R., May, G. and Bremer, E. (1988) Cell 52, 569–584.

[3] Horwitz, M.S.Z. and Lock, L.A. (1990) Prog. Nucleic Acids Res. Mol. Biol., 38, 137–164.

[4] Travers, A.A. (1987) CRC Crit. Rev. Biochem. 22, 181–219.

[5] Wang J.C. (1985) Annu. Rev. Biochem. 54, 665–697.

[6] Hulton, C.S.J., Seirafi, A., Hinton, J.C.D., Sidebotham, J.M., Waddell, L., Pavitt, G.D., Owen-Hughes, T., Spassky, A., Buc, H., Higgins, C.F. (1990) Cell 63, 631–642.

[7] Lilley, D.M.J. and Higgins, C.F. (1991) Mol. Microbiol. 5, 779–783.

[8] Pruss, G.J. and Drlica, K. (1989) Cell 56, 521–523.

[9] Borowiec, J.A. and Gralla, J.D. (1987) J. Mol. Biol. 195, 89–97.

[10] Mishra, R.K., Gopal, V. and Chatterji, D. (1990) FEBS Lett. 260, 273–276.

[11] Aubel, D.T., Allen, T.L. and deHaseth, P.L. (1986) J. Biol. Chem. 261, 11202–11206.

[12] Miller, J.H. (1972) Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

[13] Mishra, R.K. and Chatterji, D. (1991) Nucleosides Nucleotides 10, 607–608.

[14] Mishra, R.K. and Chatterji, D. (1993) J. Biosciences 18, 1–11.

[15] Mishra, R.K. and Chatterji, D. (1993) Mol. Microbiol. 8, 507–515.

[16] Margalit, H., Shapiro, B.A., Nussinov, R. Owens, J. and Jernigan, R.L. (1988) Biochemistry 27, 5179–5188.

[17] Nussinov, R. (1984) J. Biol. Chem. 259, 6798–6805.

[18] Werel, W., Schickor, P. and Heuman, H. (1991) EMBO J. 10, 2589–2594.

[19] Palecek, K.E. (1991) Crit. Rev. Biochem. Mol. Biol. 26, 151–226.

[20] Amouyal, M. and Buc, H. (1987) J. Mol. Biol. 195, 795–808.

[21] Aubel, D.T. and deHaseth, P.L. (1988) J. Mol. Biol. 202, 471–482.

[22] Jyothirmai, G. and Mishra, R.K. (1990) Proceedings of DEA Symposium on Molecular Biology of Microorganisms (BARK Symposium, Bombay), pp. 120–125.

[23] Dunaway, M. and Ostrander, E.A. (1993) Nature 361, 746–748.

[24] Higgins, C.F., Hinton, J.C.D., Hulton, C.S.J., Owen-Hughes, T., Pavitt, G-D. and Seirofi, A. (1990) Mol. Microbiol. 4, 2007–2012.

[25] van Holde, K.E.(1989) Chromatin, Springer-verlag, Berlin.