Two Distinct Curved DNAs Upstream of the Light-responsive\textit{psbA}\nGene in a Cyanobacterium

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\textit{Note}

A functional intrinsic DNA curvature, CIT, and potential DNA-binding factors for the basal transcription of\textit{psbA2} have been reported in a cyanobacterium, \textit{Microcystis aeruginosa} K-81 (Asayama \textit{et al}., \textit{Nucleic Acids Res.}, 30, 4658–4666 (2002)). In this article, we found another novel curved DNA, which was induced by RNA polymerases binding to the promoter region. Circular permutation analyses showed that the curved center of RNA polymerase-induced DNA bending (RIB) lies at approximately the +10 site, referring to the transcription start point as +1, in the RNA polymerase-DNA complex. Regions containing the curved center of RIB and CIT contributed to the basal transcription in vivo and in vitro. These results indicate that the region upstream of K-81\textit{psbA2} has two distinct curved DNAs, CIT (sequence-directed type) and RIB (protein-induced type).

Key words: transcription; RNA polymerase; sigma factor; intrinsic curvature; protein-induced DNA bending

A unicellular colony-forming cyanobacterium (blue-green algae), \textit{Microcystis aeruginosa} strain K-81 (hereafter referred to as K-81) contains a \textit{psbA2} gene encoding a D1 homolog as a core protein in photosystem II.\textsuperscript{11} It has been characterized for basal, light-responsive, and circadian-rhythmic transcription of the \textit{psbA2} gene.\textsuperscript{2–5} We have found and characterized an intrinsic (static or sequence-directed, the same meaning) DNA curvature, CIT (Changeable bending-center sites of an Intrinsic curvature under Temperature conditions) located in the 5’-upstream region (–241 to –80) of \textit{psbA2}.\textsuperscript{6} We have reported the universality of the curved DNA structure in \textit{psbA} genes of another cyanobacterium, red alga, and in plants, and also showed the roles of the curvature in basal transcription in cyanobacteria. Potential factors contributing to the basal transcription of \textit{psbA2} with the CIT curvature were also found.\textsuperscript{7} In these studies, basal (non light-responsive) transcription by RNA polymerases with principal sigma factors\textsuperscript{8–11} was observed in \textit{Escherichia coli} and cyanobacteria, and the minimal \textit{cis}-element was a region from –38 to +14.\textsuperscript{2,3} Moreover, light-responsive expression with the region from –38 to +46 was found to be controlled at the transcriptional and post-transcriptional level.\textsuperscript{1,3}

In this study, we tested whether RNA polymerase binding to the \textit{psbA2} promoter caused protein-induced DNA bending or not. A 126-bp DNA fragment amplified by PCR with the oligonucleotides GKA6 and GKA10,\textsuperscript{7} containing the \textit{psbA2} promoter region from –80 to +46, was cloned into an Sma\textsc{Bgl} II site of the plasmid pCY712 to create pGK3, which can supply a series of 526-bp permuted fragments (the length of each insert is the same, Fig. 1a) for Circular Permutation Analysis (CPA). The principle of CPA for determination of a bending-center site on a permuted fragment is that in the gel matrix of PAGE, a fragment with a bend nearer its center migrates more slowly than a fragment with a bend nearer its end.\textsuperscript{13} The respective permuted fragment was purified from the gel, 5’-end labelled with [\textsuperscript{32}P]ATP, mixed with heterologous RNA polymerase (\textit{E. coli} core + K-81 principal sigma factor \(\sigma^{A3} = \operatorname{Es}^{A4}\)) and then put through a 4% PAGE at 30°C for CPA (Fig. 1b). The 526-bp permuted EcoRI fragment was taken as a control where no RNA polymerase was added, and no complexes were observed to form (data not shown). Results showed that the position of 526-bp fragments unbound by \(\operatorname{Es}^{A4}\) remained the same in respective lanes (lanes 1 to 6), again indicating that no intrinsic DNA curvature exists in the region from –80 to +46.\textsuperscript{9,10} On the other hand, two types of DNA-protein complexes, named C1 and C2, were observed and these differed in position, demonstrating that \(\operatorname{Es}^{A4}\)-induced DNA curvatures exist as two formations of protein-DNA complexes at 30°C. To locate the curved center-site and measure the angle of the curved DNA, relative mobilities of C1 on the gel were plotted against the position of restriction enzyme-sites in the permuted fragment (Fig. 1c). The fastest and slowest mobilities

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were found in the permuted EcoRI and EcoRV fragments, respectively. The center-site of curved DNA was calculated\cite{15} to be in approximately the +10 position. Thimpon and Landy (1988) also developed an empirical relationship that relates the mobility of the fragments in CPA to the bending angle.\cite{14} This equation is \( \mu M/\mu E = \cos \alpha/2 \), where \( \mu M \) is the mobility of the fragment with the bend sequence closest to the middle (i.e. the slowest, in Fig. 1c, EcoRV), and \( \mu E \) is the mobility of the fragment that contains the bend sequence nearest to the end (the fastest, in Fig. 1c, EcoRI). When this equation was used, the angle of curved DNA with EcoRI \( \alpha \) in the C1 or C2 complex was 71 or 83 degrees, respectively. We also detected the RNA polymerase-induced DNA curvatures with very similar results when purified \( E. coli \) Eco RI and EcoRV was used. Unfortunately, with a partially purified authentic cyanobacterial RNA polymerase fraction \( ^{14}Eco \) \( ^{14}A1 \), \( ^{14}A2 \), \( ^{14}A3 \), and \( ^{14}A9 \) we have not succeeded in obtaining a clear pattern of CPM, because the high molecular mass of the DNA-RNA polymerase complex with other DNA-binding proteins resulted in a stack at the top of the polyacrylamide gel in electrophoresis. Thus we concluded that the -80/+46 region carrying the minimal cis-element from -38 to +14 for the basal transcription of \( psbA2 \) has an RNA polymerase-induced DNA bend (RIB).

To evaluate the effects of the regions containing the CIT or RIB curvature, we constructed mutants and examined the \( psbA2 \) transcription in vivo and in vitro (Fig. 2). For in vivo analyses, we prepared a series of \( lacZ \)-fusion constructs containing 5′- and 3′-deleted \( psbA2 \) promoter regions as follows. Although a recombination system has not been established in \( Microcystis aeruginosa \) K-81, basal- or light-responsive K-81 \( psbA2 \) transcription has been well studied in the recombinant cyanobacterium \( Synechococcus elongatus \) PCC 7942 (hereafter called PCC 7942) cells.\cite{3,7} DNA fragments of K-81 \( psbA2 \) were amplified by PCR with a set of primers [Fig. 2: GKA1, 5′-ttgccccgggATACACCTGCATGATC-3′ (\( Smal \) site, underline); GKA7, GKA9, GKA10, and GKA34 were previously reported\cite{13,51}] and the plasmid pHNL7-up\cite{9} as a template DNA, then these PCR products were digested with \( Smal \) and BglII. Resultant segments were inserted into an \( Smal-BglII \) site of pAM990, which is a promoter-probe vector,\cite{15} to create pAG plasmids (Fig. 2). These pAG plasmids were introduced into the genome of the cyanobacterium PCC 7942 by homologous recombination. The recombinant PCC 7942 cells were selected on BG11 liquid medium\cite{10} containing spectinomycin sulfate (40 \( \mu \)g/ml). Activities of \( \beta \)-galactosidase for the basal transcription of K-81 \( psbA2 \)\cite{3,10} were measured using cells grown under continuous white-light illumination (35 \( \mu \)E \( m^{-2} s^{-1} \)). A reduction in the activity and no activity of \( \beta \)-galactosidase was found for AG430 (+CIT) versus AG407 (−CIT), and AG429 (+RIB) versus AG410 (−RIB), respectively. This means that the regions containing the curved center contribute to the \( psbA2 \) transcription. For the in vitro assay, the multiple-round run-off assay was done as described\cite{17}.
The effects of the upstream region, involving CIT and RIB curvatures, for the basal transcription.

Each fragment containing deletion derivatives of the K-81 \( psbA2 \) upstream region was synthesized by PCR and inserted into pAM990 to create pAG for transcriptional \( \beta\)-galactosidase fusion plasmids. These constructs were introduced into a neutral site locus on the PCC 7942 genome by homologous recombination. The recombinant cells were grown continuously under white-light illumination (35 \( \mu \text{E m}^{-2} \text{s}^{-1} \)) for the measurement of basal transcription based on the \( \beta\)-galactosidase activity. The \textit{in vitro} mRNA synthesis of K-81 \( psbA2 \) mRNA by partially purified authentic K-81 RNA polymerase (*Es*)A1 with a principal sigma factor (\( \sigma_A1 \)) from the K-81 cyanobacterium is indicated by plus (+) and minus (−) signs.

Previously, the assay mixture (40 \( \mu \text{l} \)) was made of 50 mM Tris-HCl (pH 8.0), 0.05 mM EDTA:2Na, 0.5 mM DTT, 10 mM MgCl2, 0.1 mM each of ATP/CTP/GTP, 0.05 mM [\( ^{32}\text{P} \)]UTP (18.5 GBq/mmol, du Pont), linearized template pAG DNAs (1 \( \mu \text{l} \), 2 pmol), and partially purified authentic RNA polymerase *EsA1 from the K-81 strain [2.5 \( \mu \text{l} \) (about 1 pmol of RNA polymerase)]. We confirmed that the \( psbA2 \) transcript was abundant with pAG430 and decreased with pAG407. There was also apparent mRNA synthesized from pAG429 but not from pAG410 (Fig. 2). These findings supported the \textit{in vivo} results well.

On the basis of results available so far, we can consider the structure of the \( psbA2 \) upstream region. A model is shown in Fig. 3. This region potentially has CIT, and then RIB is eventually induced by RNA polymerase. Structural changes of DNA distortion by DNA-RNA polymerase interaction at the promoter influence initiation kinetics for close/open complex formation in transcription. It has been generally accepted that when the bacterial RNAP holoenzyme (core enzyme + principal sigma factor) binds to the promoter DNA, it usually covers 75–80 bp (between positions −55 and +20) and approximately 14 bp of the promoter DNA (between positions −11 and +3) are melted, yielding the transcription bubble, in the step with an open binary complex. The protein-induced DNA bends by RNA polymerases have been sometimes reported at the core promoter region, \textit{i.e.} gal operon\(^{17}\) and ctRNA II.\(^{18}\) These bends might result in a DNA wrapping around the RNA polymerase with sigma factors on promoters.\(^{19}\) RNA polymerase-induced change in the sense of the superhelix from right-handed to left-handed might result in a local unwinding of DNA for open complex formation during transcription.\(^{20}\) The studies using insertion DNA of rightward spaced-curve or plane-curve the adjacent upstream \( \beta\)-lac-
tamase gene promoter indicated that the binding affinity of RNAP was changed by those insertion, and that an increased activity of DNA melting at the positions $\pm 11(T)/\pm 10(G)$ and $\pm 3(T)/\pm 4(A)$, which are edges in the melting region with RNA polymerase, appeared when the rightward spaced-curve was used rather than the plane-curve. Therefore, the right-handed superhelical writhe facilitates transcription when compared with the effect produced by a straight DNA segment. Intrinsic DNA curvature and/or DNA wrapping surrounding RNA polymerase apparently contributes to the transcription initiation. It is also generally agreed that protein-induced DNA bending caused by positive or negative regulators with RNA polymerase plays significant roles in transcription.

Not only bacterial sigma70-RNA polymerase but also archaeal and eukaryotic RNA polymerase interact with promoter DNA boundaries, as given by data on its crystallographic three-dimensional structure. For instance, a phage type RNA polymerase consists of single catalytic subunit and does indeed bend its promoter DNA. The bend in the T7 RNA polymerase complex is predicted to be about 40–60 degrees and to be centered around positions -2 to +1, at the start site for transcription, while the intrinsic bend is much smaller (about 10 degrees).

Recent results showed that E. coli RNA polymerase $\sigma^70$ can specifically recognize in vitro the $psbA2$ promoter and binds to an anomalous large segment (from -115 to +23) of the sequence upstream of $psbA2$. Our findings in this study might be unique, in that a single promoter region has two distinct types of DNA curvatures, one a sequence-directed CIT and the other a protein-induced RIB. This also presents a novel feature of RNA polymerase induced-DNA bend the center of which is located downstream (+10) from the transcription start point (+1). These experiments are also the first case of characterizing cis-elements involving two curved DNAs around a single promoter in photosynthetic organisms. It is still unclear whether the RIB of $psbA2$, the center of which lies around the +10 site within the minimal promoter region from -38 to +14, affects the open complex DNA formation. Because potassium permanganate (KMnO$_4$) footprinting analysis is useful for detecting promoter DNA melting approach with the RIB mutants will be helpful in characterizing the RIB function involving a DNA configuration change.

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References