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Different superstructural features of the light responsive elements of the pea genes *rbcS-3A* and *rbcS-3.6*

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Analyses of the DNA sequences, named Light Responsive Elements (LREs), relevant in the photoactivation of the two pea genes *rbcS-3A* and *rbcS-3.6*, encoding the same protein but differently expressed, have been carried out, taking advantage of two complementary methods: a theoretical analysis, based on conformational energy calculations, and an experimental evaluation of LREs curvature, derived from gel electrophoretic mobilities of multimers of the LREs and of oligonucleotides corresponding to the three boxes in which they can be dissected. Theoretical and experimental analyses show that the curvature of the *rbcS-3A* LRE is larger than that of *rbcS-3.6* LRE, and seems to be correlated with *rbcS-3A* higher transcription efficiency.

Light responsive element; DNA superstructure

1. INTRODUCTION

An important role, either in interactions between regulatory proteins and specific DNA sequences, or in allowing interactions between RNA polymerase and transcription factors, has been recently associated with DNA curvature and/or curvability [103]. Therefore, the analysis of curvature and curvability of DNA regions controlling gene expression appears useful to screen DNA sequences that are better candidates for specific interactions with proteins, as well as to obtain information on the role of the nucleotide sequences in the recognition mechanism with protein factors.

An original method for predicting the curvature of nucleotide sequences was recently developed by De Santis and co-workers [4–6]. We have applied this theoretical method to predict the curvature of DNA sequences involved in the light regulation of transcription of the pea genes *rbcS-3A* and *rbcS-3.6*, which code for the same protein (the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase) but are differently expressed (*rbcS-3A* contributes 40% of the total transcript, *rbcS-3.6* less than 7%) [7]. These sequences, called light responsive elements (LREs) positioned between −170 and −110 from the start-site of transcription, are highly conserved in all pea *rbcS* genes [7] and bind to a protein factor, GT1, which is required for the light- regulated expression of *rbcS* genes [8,9].

To experimentally assay the curvature of the LREs, we have synthesized the LREs and oligonucleotides corresponding to the three boxes in which they are dissectable on the basis of biochemical analyses [7,8], and measured their gel electrophoretic mobilities after ligation. This approach allows multimers to be obtained, with defined superstructural features.

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2. MATERIALS AND METHODS

Oligodeoxyribonucleotides were synthesized on a Biosearch 8600 DNA synthesizer, purified and phosphorylated as previously described [6,10]. Phosphorylated oligonucleotides were heated to 60°C, slowly cooled to form hybrids, and ligated to obtain polynucleotides. Ligated products were run, at room temperature, on non-denaturing 10% polyacrylamide gels. The applied voltage was 4 $V \cdot cm^{-1}$.

3. RESULTS AND DISCUSSION

Fig. 1 shows the curvature profiles from −300 to the start-site of transcription, reported as the modulus of the curvature vector $|C|$ vs the nucleotide sequence.

The LRE regions show noticeably different curvature features (Fig. 1a,b), namely in the case of *rbcS-3A* the profile presents two maxima connected by a region of lower curvature, while in the case of *rbcS-3.6* the overall curvature appears lower and broader.

To experimentally assay these findings, we synthesized the LREs of the two genes and oligonucleotides corresponding to the sequences box I, box II, and box III; we then measured their electrophoretic mobilities as well as those of their multimers, having the phase of B-DNA. The retardation of the multimers (ratio between the apparent and real molecular weight) allows evaluation of local DNA curvature, as described in pre-

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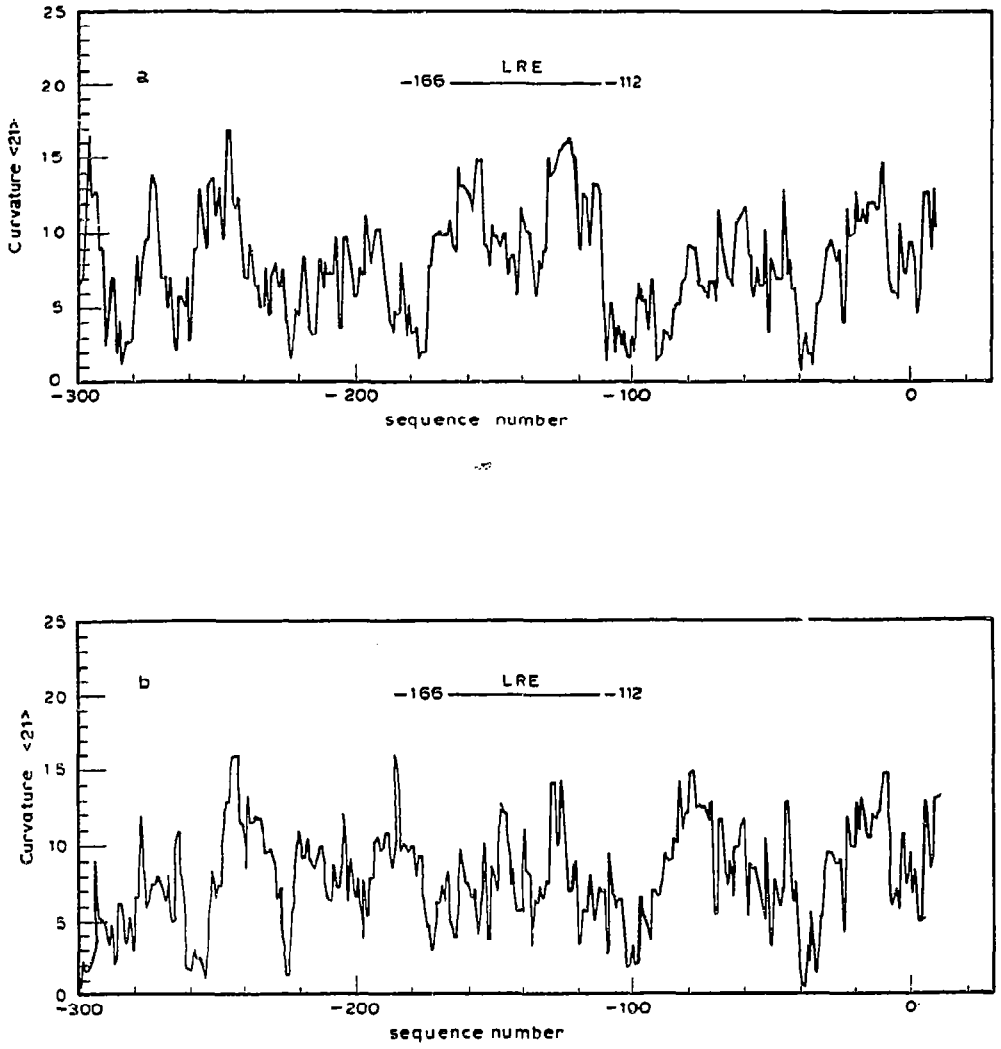


(c)

-180 -111

3A TAAGGCTAGCACACAAAATTTCAAATCTTGTGTGGTTAATATGGCTGCAAACTTTATCATTTCACTATC
3.6 TAAGGTTAGGACACACAACTTTTCAATCTTGTGTGGTTAATATGGCTGCAAAGTTTATCATTTCACAATC

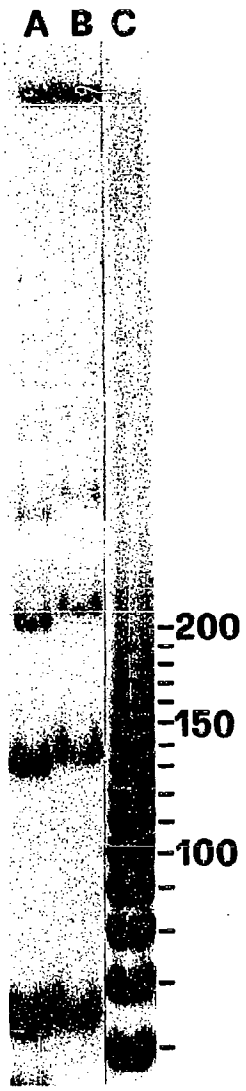
-110 -41

3A TAACAAGATTGGTACTAGGCAGTAGCTAAGTACCACAATATTAAGACCATAATATTGGAAATAGATAAAT
3.6 TAACAAGATTGGTACTAGGCAGTAGCTAATTACCACAATATTAAGACCATAATATTGGAAATAGATAAAT

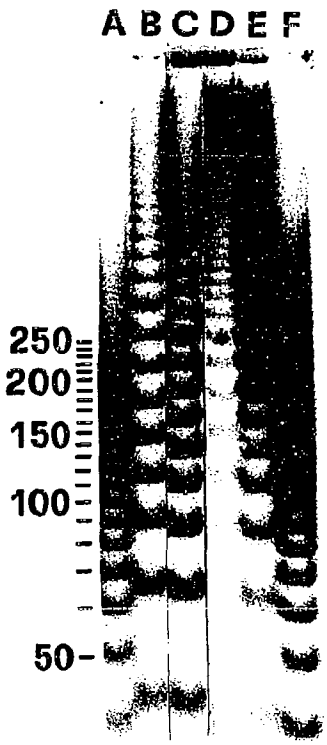
-40 +10

3A AAAAACATTATATATAGCAAGTTTTAGCAGAAGCTTTGCAATTCATACAG
3.6 AAAAACATTATATATAGCAAGTTTTAGCAGAAGCTTTGCAATTCATACAG

Fig. 1. Theoretical curvature profiles, averaged over 21 nucleotides, of the 300 bp upstream regulatory regions of the pea genes *rbcS-3A* (a), and *rbcS-3.6* (b), versus the sequence number. In (c) are reported the 5′ nucleotide sequences, from −180 to +10, of the two genes. Boxes I, II and III are underlined.



a



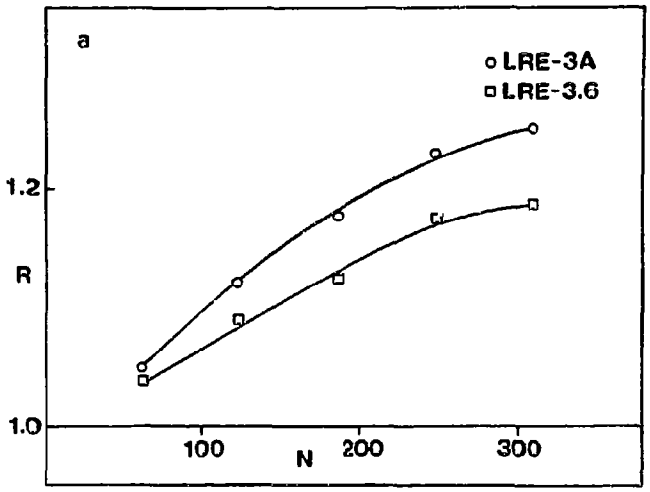
b

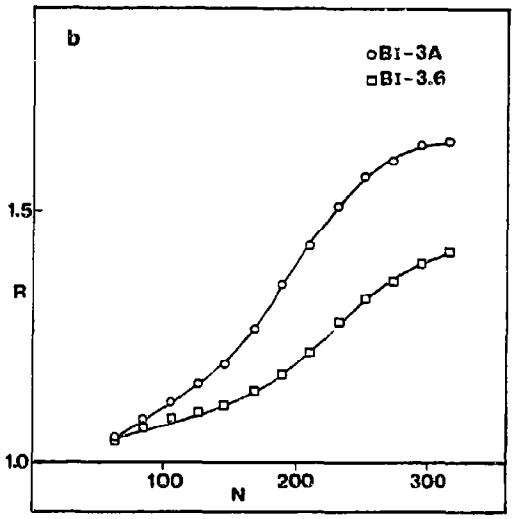
Fig. 2. Autoradiogram of the multimer series derived from ligation of the monomers reported in Table I. (a) LREs. Asterisks indicate multimers of 186-bp in the case of LREs and 190-bp in the case of NBH. Lane A, LRE-3.6; lane B, LRE-3A; lane C, NBH. (b) Boxes. Asterisks and stars indicate respectively multimers of 180-bp and 210-bp sequence length. Lanes A and F, NBH; lane B, BI-3A; laneC, BI-3.6; lane D,BIII-3A; lane E, BIII-3.6.

vious papers [6,10]. The synthesized oligonucleotides are described in Table I. The sequence NBH (Not Bent Hagerman) was synthesized and ligated for use as a standard of straight DNA [11].

Figure 2 shows a typical polyacrylamide gel, where the anomalous electrophoretic behaviour of (a) the LREs, and (b) sequences corresponding to boxes I and III, with respect to the non-curved sequence NBH, is evident. In Fig. 3a,b,c the retardations of all the examined sequences derived from the reported as well as many others experiments, are presented as R, the ratio of apparent and real number of base pairs versus N, the actual chain length; R values resulted in satisfactory agreement with the theoretical prediction. The LREs have superstructural features more evident in the case of the *rbcS-3A* gene. The difference in the curvature of the 2 genes appears more relevant when box I and box III sequences are considered, while the box II region, which has the same sequence in the 2 genes, can be considered straight. The reported results show the possibility of studying the superstructural features of DNA in the regulatory regions where DNA curvature can be in relation with the recognition of proteins, using theoretical calculations and electrophoretic mobilities of synthetic oligonucleotides, model of the DNA tract of interest.

The LRE of the *rbcS-3A* gene shows a well-defined superstructure, characterized by 3 differently curved tracts of about 20 nucleotides long, well correlated with results of biochemical and genetic analyses [8]. Namely, box II, whose sequence does not suffer mutations, appears as a straight DNA tract. Box II is, probably, involved in direct recognition with GT1 factor. Box I and box III show a noticeable curvature, that could facilitate the wrapping of DNA around the protein factor, positioned on box II and box III. A similar schema of interactions has recently been proposed for the binding of the bacterial repressor Lex A to the *caa* gene operator





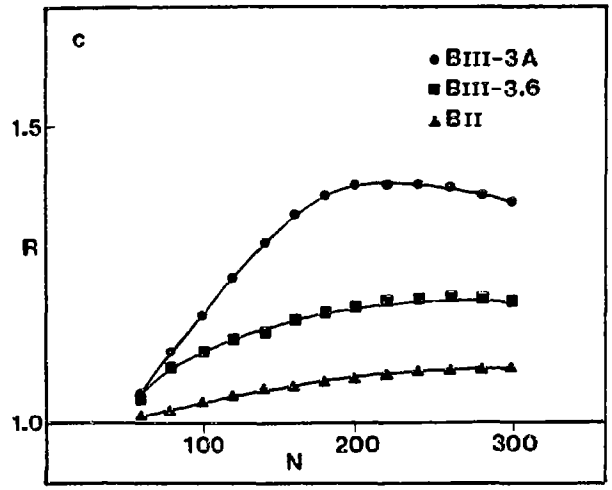


Fig. 3. Multimer mobilities, R, determined from comparison with the electrophoresis markers, are plotted as function of N (the actual chain length). Sequences of repeating units in the multimers are shown in Table I. Sequences corresponding to: (a), LREs; (b), box I; and (c), boxes II and III.

[12]. Furthermore, these results suggest that the different expression of the two genes *rbcS-3A* and *rbcS-3.6* could be correlated with the different superstructural features of the LREs, since the higher curvature of box I and box III in the *rbcS-3A* gene can give rise to a higher association constant between the LRE and the transcription factor GT1.

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Table I

Sequences examined. Only one strand of the duplex is shown. The duplexes were constructed with 3-bp protruding 5'-ends. The names of the sequences identify the corresponding regions on the genes (i.e. BI-3A corresponds to box I of *rbcS-3A*). BII has the same sequence in the two genes.

Name	Sequence (5'-3')
rbcS-3A	
LRE-3A	ACACAAAATTTCAAATCTTGTGTGGTTAATATGGCTGCAAACTTTATCATTTTCACTATCTA
BI-3A	CACACAAAATTTCAAATCTTG
BIII-3A	TGCAAACTTTATCATTTTCA
rbcS-3.6	
LRE-3.6	ACACACAACTTTTCAATCTTGTGTGGTTAATATGGCTGCAAAGTTTATCATTTCACAATCTA
BI-3.6	ACACACAACTTTTCAATCTTG
BIII-3.6	CTGCAAAGTTTATCATTTCA
BII	CTTGTGTGGTTAATATGGCT
NBH	GGGTCGACCC

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