ELSEVIER

Biophysical Chemistry 53 (1995) 241–245

Biophysical Chemistry

Differential binding of RNA polymerase to the wild type Mu mom promoter and its C independent mutant: a theoretical analysis

T. Murlidharan Nair [a], B.D. Kulkarni [a,*], V. Nagaraja [b]

[a] *Chemical Engineering Division, National Chemical Laboratory, Pune 411 008, India*
[b] *Center for Genetic Engineering, Indian Institute of Science, Bangalore 560 012, India*

Received 5 May 1994; accepted in revised form 21 September 1994

Abstract

Using the theoretical model for DNA bending we have analyzed the Mu mom promoter wild type and its mutant tin7 which showed differential binding to the RNA polymerase. We have demonstrated here the structural change as a result of the point mutation which may be responsible for the altered binding of RNA polymerase. Analysis using both sets of parameters essentially gives the same result.

Keywords: DNA curvature; Promoter structure; RNA polymerase

1. Introduction

The fundamental processes that occur inside cells (packaging, replication, recombination, restriction and transcription) all involve a complex interplay of protein–DNA interactions. The structural, biochemical, and molecular genetic studies of protein–nucleic acid complexes have revealed two main sources of sequence specificity in protein nucleic acid interactions: (i) direct hydrogen bonding and van der Waal's interaction between the protein side chain and the exposed edges of base pairs and (ii) the sequence dependent bendability or deformability of duplex DNA or RNA (i.e. certain nucleic acid sequences take up a particular structure required for binding to a protein at lower free energy that other sequences) ([1] and references therein).

The overall structure of DNA, being sequence dependent, has been the subject of intense theoretical and experimental investigations and is now a well established fact [2–4]. The ability of protein to discriminate between different regions of the DNA molecule depends first on the fact that the sequence of such a molecule is not uniform [5] and this is directly reflected in its local structure. The sequence-dependent deformability of duplex DNA or RNA that provides specificity for sequences being recognized by protein can include the melting of base pairs. Sequence recognition by protein is also attributed to the sequence dependent ability of single stranded nucleic acid to take up the conformation required for protein binding. The distortions pro-

* Corresponding author.

SSDI 0301-4622(94)00116-2

duced in nucleic acids has an indirect role in sequence specificity, this is because: (i) the nucleic acid conformation to which the proteins bind is altered after binding and is different from its uncomplexed solution conformation, and (ii) the free energy cost associated with various nucleic acid sequences to assume a particular conformation for binding to a specific protein is different for different sequences [1].

One of the most important events wherein protein–DNA interaction plays an important role is the process of transcription. Balke et al. [6] have experimentally investigated the binding of RNA polymerase in the case of the bacteriophage Mu, which controls an unusual DNA modification function encoded by the mom gene. The mom gene is located in an operon that consists of two overlapping genes. The transcription of the phage Mu com/mom operon is trans-activated by another phage gene product, C, a site specific DNA binding protein. The mechanism by which C activates transcription has been studied earlier [6]. Our main interest in studying the aspect of differential binding of RNA polymerase was to look at the structural changes that would occur in the Mu mom promoter region due to a single-base substitution. We present here our analysis using the theoretical model wherein we have analyzed the structural changes using both the set of parameters (i) obtained by Trifonov and co-workers by fitting experimental data [7] and (ii) obtained by De Santis and co-workers by energy calculation [8–10]. The two approaches yield quite different values for roll, twist and tilt, yet both parameter sets appear to fit the experimental data reasonably well. These models have been previously used in analyzing the superstructures associated with different segments of DNA and have also been experimentally validated [8,9].

2. Experimental

2.1. Analysis using the experimentally determined parameters

We have used the CURVATURE programme [11], which uses the experimentally determined wedge angle [7] to calculate the curvature map and the DNA path of the Mu mom promoter and it mutant tin7. The programme has been specially modified by Shpigelman and Trifonov to study structural changes caused by such point mutations. The modified programme permits the projection of fragments on the same plane rather than on the maximum curvature plane where the comparison of fragments is not possible. Both the fragments are projected on the same plane (XY).

2.2. Analysis using the energy minimized values

The theoretical model for the sequence dependent superstructures of DNA was proposed by De Santis et al. [8] on the basis of conformational energy calculations. The model has been used to obtain the structural deviations from the canonical B-DNA structure in terms of the curvature vector $C(n,v)$ which represents in the complex plane (in modulus and phase) the directional change of the double helix axis between sequence number n and $n+v$. Whilst one may refer to the original work of De Santis et al. for details of the calculations [12,13], we state that the calculations require knowledge of $v^0 = 10.4$ which is the average periodicity of the DNA, $d_j = (\rho_j - i\tau_j)$ representing the orientational deviations of the jth base pair average plane from the canonical B-DNA. ρ_j and τ_j are the roll and tilt angles, respectively, obtained by energy minimization [8]. Curvature is expressed by a pair of diagrams where both the modulus and the relative phase of the curvature vector $C(n,v)$ are reported versus the sequence number n. In our calculations we have assumed $v = 31$ (i.e about 3 turns of the DNA helix) and the value of curvature is assigned to the center of the sequence tract considered. The curvature dispersion σ^2 was also calculated following De Santis et al. [9].

3. Results and discussion

We have used theoretical models for DNA curvature to understand the superstructural features associated with the Mu mom promoter region and its mutant. Experimental studies reported by Balke et al. [6] have revealed that in vivo and in the absence of C (a transacting factor), RNA polymerase bound the wild type promoter region at a site designated P2

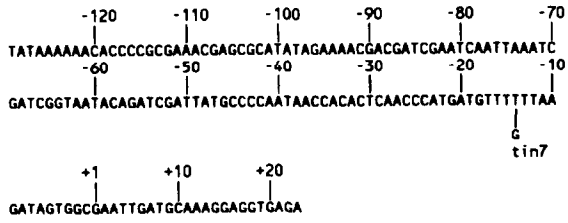

Fig. 1. Sequence of the Mu mom promoter region. The tin7 mutation is a T to G substitution at position −14 with respect to the transcription start site (see Fig. 1 of Ref. [6]).

(see Fig. 1). The P2 region was shown by DNase I-footprinting to extend from −74 to −24 with respect to the transcription initiation. A known C binding site −35 to −54 falls within this region. In the presence of C in vivo, RNA polymerase bound the wild type promoter region at a site designated P1. The P1 region was shown to extend from −56 to +21 by DNase I-footprinting. The analysis of the C independent mutant (which initiated transcription at the same position as that of the wild type) revealed that in vivo in the absence of C, RNA polymerase bound mutant tin7 (which contains a T to G substitution at −14) predominantly at P1 [6]. These observations point to the fact that the T to G substitution at position −14 in the Mu mom promoter alters the signature (in terms of it structure) which the RNA polymerase is unable to recognize.

The structural deformations associated with the upstream region of the mom gene have been investigated using nearest neighbor models which take into account the influence of the flanking sequences on the overall structure. The overlap of the curvature map of the Mu mom promoter and its mutant is shown in Fig. 2. The curvature, given in DNA curvature units [14], has been calculated using the experimentally determined 16 wedge angles. A steep drop in the curvature value is seen in the vicinity of the mutation; this necessarily means a change in the shape of the DNA. The other region shows a perfect overlap of curvature. The substitution of T by G at the −14 position in the mom promoter produces a TG step (see Fig. 1). The TG step kills the T runs thus severing local curvature. It also changes, at this point, the direction of the DNA axis. TG stack also adds flexibility, thus changing the binding constant [15]. A clearer perspective of the change in shape

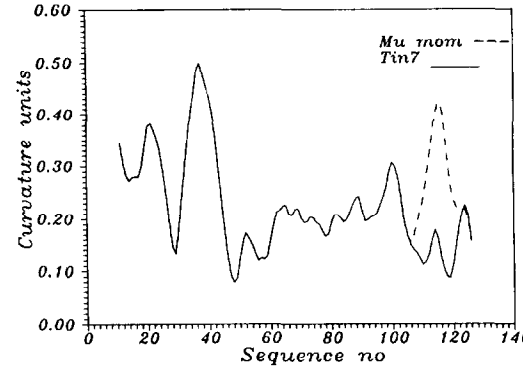

Fig. 2. Curvature map of the mom promoter and its mutant tin7 showing the separated and overlapping region. Curvature is given in DNA curvature units which is the average curvature of DNA in the nucleosome core particle, 1/42.8 Å (see Ref. [14]).

can be obtained from the DNA path. The overlap of the DNA path of the wild type promoter and its mutant is shown in Fig. 3. Both these fragments have been projected on the same plane to facilitate comparison. The deviation of the DNA axis in the vicinity of the mutation can be clearly seen and may be responsible for the altered recognition.

We obtained essentially similar results with the energy minimized parameters obtained by De Santis et al. This model has been very successful in predicting the gel electrophoresis retardation due to point mutations in a tract of SV40 DNA [12] which was experimentally investigated by Milton et al. [16]. Because the model was very successful in predicting gel electrophoretic retardation changes induced in a tract of 173bp by a single-base substitution we also

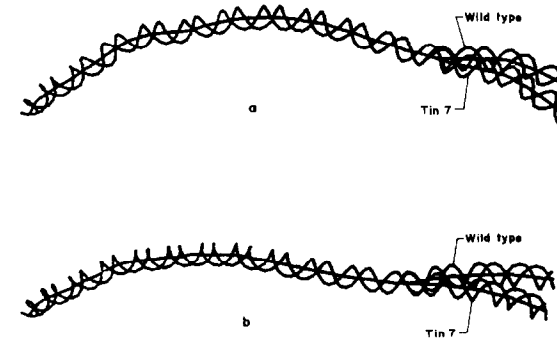

Fig. 3. Overlap of the DNA path of the mom promoter and its mutant. (a) Calculated using experimentally determined parameters. (b) Calculated using the energy minimized values.

used it to study structural changes induced in the Mom promoter caused by a single-base substitution. Fig. 4 shows the overlap of the curvature profile, phase and curvature dispersion of the mom promoter and its mutant. The curvature dispersion (σ^2) easily allows the individual recognition of the sequences of higher curvature. The DNA path calculated using the energy minimized values of the DNA parameters is similar to that obtained with the parameters obtained by experimental calculations and also shows a deviation in the DNA axis.

The results of our analysis using both sets of parameters suggest that there is definitely a change in the DNA superstructure due to a single-base substitution. As a consequence of this, the relative amplitude of the DNA grooves are changed with respect to the canonical B-DNA. This results in the development of a differential electrostatic field along the curved DNA tracts, changes in hydration and, finally, in affinity towards proteins.

Further in conclusion we would like to stress that the theoretical models based on the simple nearest-neighbor interaction hypothesis are capable of translating the deterministic fluctuations of the base sequences into information on DNA superstructure. Similar deviations of the DNA axis due to a single-base substitution are predicted by both sets of parameters even though the values appear to be different. This may be due to the fact that in order to calculate the preferred configuration of a DNA molecule, the important parameter is not the absolute value for an individual base step but the relative differences be-

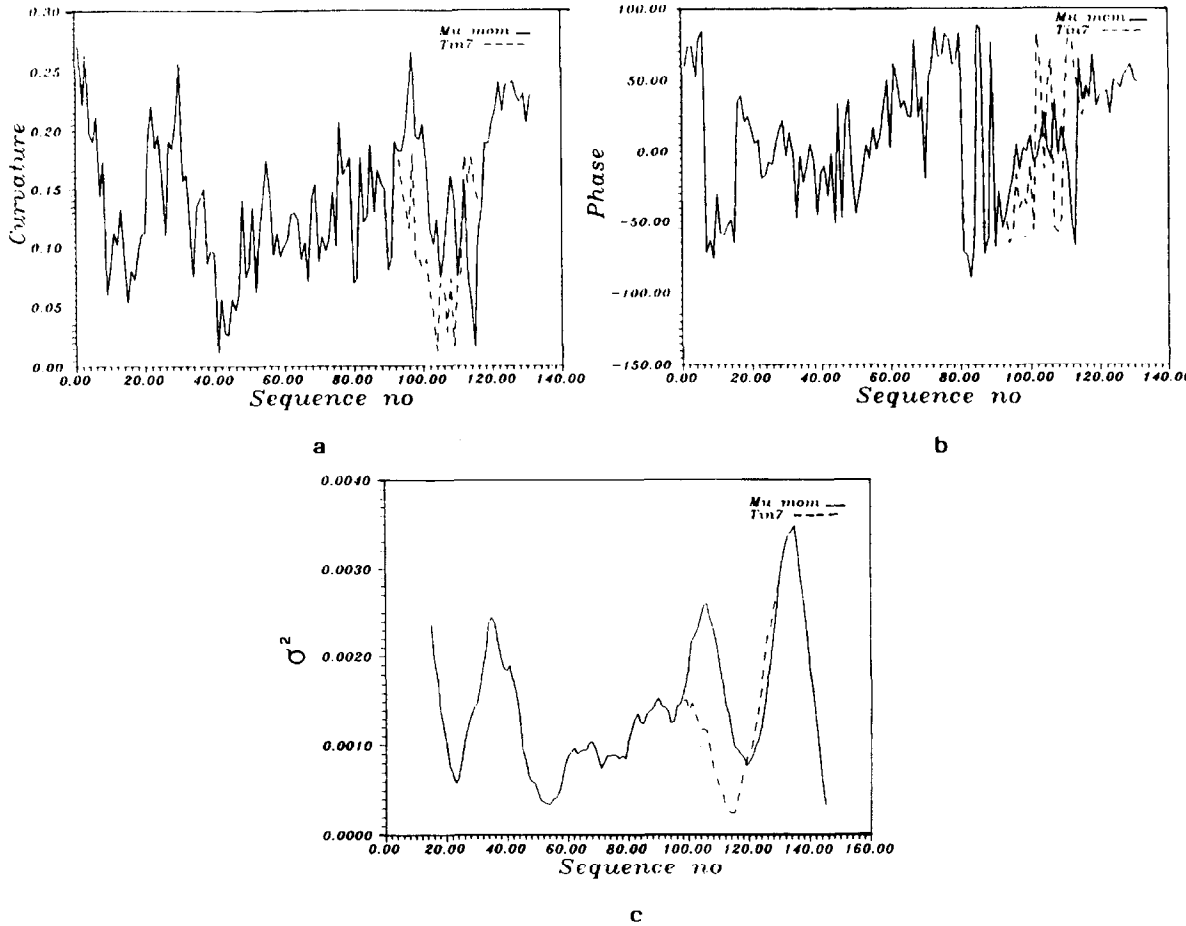

Fig. 4. Overlap of: (a) curvature profile, (b) phase and (c) curvature dispersion σ^2 for the mom promoter and its mutant.

tween base steps. We would further like to suggest that this change in the shape of the DNA contributes to changes in the complex array of factors discussed above which in turn may be responsible for the altered binding of RNA polymerase.

Acknowledgements

We would like to thank Drs. Ed Shpigelman and E.N. Trifonov for tailoring the CURVATURE programme according to our requirements. TMN thanks Prof. Trifonov for useful discussions. The authors also thank Prof. P. De Santis for helping us in the initial stages of this study to understand his model. This work was supported by the Council of Scientific and Industrial Research India.

References

[1] T.A. Steitz, Quart. Rev. Biophy., 23 (1990) 205.
[2] J.C. Marini, S.D. Levene, D.M. Crothers and P.T. Euglund, Proc. Natl. Acad. Sci. USA, 79 (1982) 7664.
[3] L.E. Ulanovsky and E.N. Trifonov, Nature, 326 (1987) 720.
[4] L.E. Ulanovsky, M. Bodner, E.N. Trifonov and M. Choder, Proc. Natl. Acad. Sci. USA, 83 (1986) 862.
[5] A. Travers in DNA–Protein Interaction, Chapman and Hall, London, 1993, p. 52.
[6] V. Balke, V. Nagaraja, T. Gindlesperger and S. Hattman, Nucl. Acids Res., 20 (1992) 2777.
[7] A. Bolshoy, P. McNamara, R. Harrington and E.N. Trifonov, Proc. Natl. Acad. Sci. USA, 88 (1991) 2312.
[8] P. De Santis, A. Palleschi, M. Savino and A. Scipioni, Biophy. Chem., 32 (1988) 305.
[9] P. De Santis, A. Palleschi, M. Savino and A. Scipioni, Biochemistry, 29 (1990) 9269.
[10] P. De Santis, S. Morositti, A. Palleschi and M. Savino, in E. Clementi and S. Chin (Editors), Structures and Dynamics of Nucleic Acids, Proteins and Membranes, Plenum, New York, 1986, p. 31.
[11] E.S. Shpigelman, E.N. Trifonov and A. Bolshoy, CABIOS, 9 (1993) 435.
[12] P. De Santis, A. Palleschi, M. Savino and A. Scipioni, Biophy. Chem., 42 (1992) 147.
[13] D. Boffelli, P. De Santis, A. Palleschi, M. Savino and A. Scipioni, Int. J. Quant. Chem., 42 (1992) 1409.
[14] E.N. Trifonov and L.E. Ulanovsky, in R.D. Wells and S.C. Harvey (Editors), Unusual DNA Structures, Springer Verlag, New York, 1988, pp. 173–187.
[15] P.T. Mc Namara, A. Bolshoy, E.N. Trifonov and R.E. Harrington, J. Biomol. Struct. Dyn., 8 (1990) 529.
[16] D.L. Milton, M.L. Casper and R.F. Gesteland, J. Mol. Biol., 213 (1990) 135.