CONTEXT-DEPENDENT EFFECTS
OF UPSTREAM A-TRACTS ON PROMOTER
ELECTROSTATIC PROPERTIES AND FUNCTION

Kamzolova S.G., Osypov A.A.*, Dzhelyadin T.R., Beskaravainy P.M., Sorokin A.A.
Institute of Cell Biophysics, RAS, Pushchino, Moscow region, Russia

* Corresponding author: e-mail: ao@icb.psn.ru

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SUMMARY

Motivation: Analysis of electrostatic properties of promoter DNA is a promising means for yielding information about promoter recognizable elements and their functioning.

Results: Electrostatic potential distribution of synthetic consensus-like promoters and their derivatives containing A-tracts at different positions in upstream region of promoter DNA was calculated and analyzed in respect with their functional behavior. Specific electrostatic motifs found in the upstream region of A-tracts containing promoters were shown to be involved as signal elements in differential recognition of the promoters by RNA polymerase α-subunit acting at early steps of complex formation.

Availability: electrostatic potential distribution analysis software is available at request to academic users (lptolik@icb.psn.ru).

INTRODUCTION

Here, electrostatic properties of three synthetic promoters P_s1, P_s2, P_s3 and their derivatives Ps1/A3-40, Ps1/A3-44 and Ps1/A3-48, containing 3 A-tracts at different positions in upstream region of Ps1 were studied. All these promoters have been earlier characterized in details in comparative experiments by their interaction with RNA polymerase at different steps of complex formation and transcription initiation (Ellinger et al., 1994a, b). The choice of these promoters for our study was motivated by their unusual functional characteristics differing from “consensus sequence rule” behavior thus stimulating a search of new promoter determinants. The results obtained in our work indicate that electrostatic characteristics of promoter DNA can be responsible for the interaction with RNA polymerase acting at early steps of complex formation.

METHODS

Three synthetic promoters P_s1, P_s2, P_s3 and their biochemical characterization were taken from (Ellinger et al., 1994a). Three derivatives of P_s1 promoter Ps1/A3-40, Ps1/A3-44 and Ps1/A3-48, containing three phased A-tract sequences located at different positions in upstream region were taken from (Ellinger et al., 1994b). Their functional characterization are presented in accordance with the data (Ellinger et al., 1994b). The electrostatic potential distribution around double-helical DNA of the promoters was calculated by the Coulomb method (Kamzolova et al., 2000) using the computer program of Sorokin A. (lptolik@icb.psn.ru).
RESULTS AND DISCUSSION

The promoters $P_{s2}$ and $P_{s3}$ have consensus sequences in -10 and -35 regions, also $P_{s3}$ has consensus 17 bp spacer between them. In $P_{s2}$ it is 16 bp length due to 1 bp deletion of $P_{s3}$ in -14 position. Thus, sequences of $P_{s2}$ and $P_{s3}$ are identical except this deletion. The $P_{s1}$ has -35 consensus and 17 bp spacer but its -10 hexamer differ from consensus at -12. The homology scores for $P_{s1}$, $P_{s2}$ and $P_{s3}$ are 59 %, 61 % and 71 %, respectively. All the three specify the correct initiation of the expected transcript in vivo. But their strengths (3.4; 8.4 and 2.1 for $P_{s1}$, $P_{s2}$ and $P_{s3}$ (Ellinger et al., 1994a)) do not correlate with the match of the promoter sequences to the consensus pattern. $P_{s3}$ with the highest homology score is the weakest with only one-quarter of the activity of $P_{s2}$. $P_{s1}$ and $P_{s2}$ with similar homology scores differ by a factor of 2.5 in activity. Also activities of $P_{s1}$, $P_{s2}$ and $P_{s3}$ are determined by different rate limiting steps within the pathway of RNA polymerase-promoter interaction: $P_{s1}$ is rate-limited during early phase of the process when the enzyme binds to the promoter, $P_{s2}$ and $P_{s3}$ are limited in a late step involving promoter clearance in transcribing complexes.

Electrostatic profiles of $P_{s2}$ and $P_{s3}$, that are limited in late steps of productive complex formation are very similar whereas $P_{s1}$ which is rate-limited during initial binding of RNA polymerase to the promoter is characterized by quite different electrostatic pattern (Fig. 1b). Because electrostatic interactions contribute to promoter activity at the very early steps of RNA polymerase-promoter recognition (Kamzolova et al., 2000), it is reasonable to suggest that variations in functioning of $P_{s1}$ as compared with $P_{s2}$ and $P_{s3}$ can be at least partly due to the difference in their electrostatic properties. Then, in the case of $P_{s1}$, electrostatic component may play a role in specifying the pathway of the interaction of the promoter with RNA polymerase as well as in determining its strength. In the case of $P_{s2}$ and $P_{s3}$, which are characterized by the same type of RNA polymerase-promoter interaction and very similar electrostatic patterns, some other factors can be responsible for the unpredictable difference in their activities, like different spatial arrangement of recognizable modules in the two promoters (16 bp spacing for $P_{s2}$ and 17 bp spacing for $P_{s3}$) leading to overstabilization of open complexes with a lower productivity at one of them ($P_{s3}$) (Ellinger et al., 1994a).

It was shown that A-tracts inserted into upstream region of $P_{s1}$ promoter can influence its function by increasing promoter activity due to facilitated RNA polymerase binding in the presence of A-tracts via some additional contacts between UP-region and $\alpha$-subunit (Ellinger et al., 1994b), but mechanisms of such interaction remain unknown.

Since electrostatic properties of promoter DNA were shown to be important for the interaction with $\alpha$-subunit (Kamzolova et al., 2000; Kamzolova et al., 2005) we decided to study how the insertion of A-tracts in upstream region of $P_{s1}$ could influence its electrostatic pattern and to analyze it in respect to the functional consequences.

Three derivatives of $P_{s1}$ containing 3 phased five-member A-tracts located at different positions in upstream region of the promoter were used (Fig. 2a). The first A-tract is centered around positions -40, -44 and -48 in promoter $P_{s1}/A_{s1}(-40)$, $P_{s1}/A_{s1}(-44)$ and $P_{s1}/A_{s1}(-48)$, respectively. $P_{s1}$ activity was shown to be stimulated by A-tracks in all three constructs. The strengths correspond to 3.4, 17.1, 10.6 and 10.8 for $P_{s1}$, $P_{s1}/A_{s1}(-40)$, $P_{s1}/A_{s1}(-44)$ and $P_{s1}/A_{s1}(-48)$, respectively (Ellinger et al., 1994b). Maximal activation (fivefold) was observed for $P_{s1}$ containing A-tracks at position -40. It should be noted that the stimulating effect was the same for $P_{s1}/A_{s1}(-44)$ and $P_{s1}/A_{s1}(-48)$ which are characterized by almost half turn dislocation of A-tracks with respect to $P_{s1}$ core promoter sequence thus indicating no determinant role of A-tract induced DNA bending in activation of these promoters.
The insertion of A-tracts in any position in upstream region of $P_{s1}$ strongly influences electrostatic properties of the promoter introducing many changes in its electrostatic pattern (compare Fig. 1b, curve $P_{s1}$ and Fig. 2b). It is noteworthy that electrostatic changes cover many sequences including those that are very far apart: the A-tracts are inserted upstream from position -40 and changes in the electrostatic profiles are observed in core sequences and downstream from the transcriptional start. The results indicate that there is no direct correlation between nucleotide sequence and its electrostatic pattern thus confirming independent character of promoter determinants based on electrostatic characteristics of promoter DNA and its structure. Electrostatic properties of DNA in far upstream region corresponding to -75 - -100 bp positions (indicating by vertical lines in Fig. 2b) are of most interest for our task since this region is known to be involved in electrostatic interaction with RNA polymerase $\alpha$-subunit (Kamzolova et al., 2000; 2005). Fig. 2b shows that $P_{s1}/A3$-44 and $P_{s1}/A3$-48 constructs which are characterized by the same activation in response to the insertion of A-tracts, exhibit electrostatic patterns similar in design in the far upstream region. The important feature of this pattern is a continuous rise of electrostatic potential at -80 bp - -90 bp with extended positive peak in this region.
A distinctly different electrostatic element is found in the far upstream region of Ps1/A3-40 which is characterized by a much more stimulating effect in response to the insertion of A-tracts. Its specific feature is a more negatively charged character of –80 - -90 bp region as compared with the adjacent site located further upstream.

As shown in the cases of T4 phage promoters, the presence of different electrostatic elements in this region is essential for different type of their interaction with α-subunit thus providing a differential response in promoter functioning (Kamzolova et al., 2000; 2005).

Thus, electrostatic patterns of the three A-tracts containing promoters can be specified according to the presence of some functionally important distinctive motifs which may be involved in differential recognition of the promoters by RNA polymerase α-subunit thus accounting for the difference in their functional behavior.

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