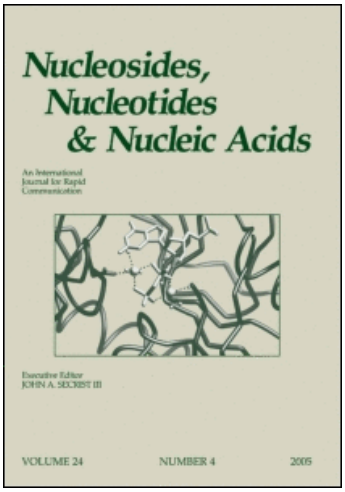

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Localization of The Initiation Site in T7 RNA Polymerase by Affinity Labeling

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LOCALIZATION OF THE INITIATION SITE IN T7 RNA POLYMERASE BY AFFINITY LABELING

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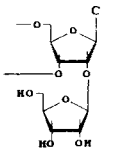
ABSTRACT: The oligonucleotide duplexes containing modified CMP residues with the additional β-D-ribofuranosyl group were used to study the interactions between T7 RNA polymerase (T7 RNAP) and its promoter. Wild-type enzyme did not undergo the affinity labelling with these reagents. However the use of Y639K T7 RNAP mutant resulted in the specific covalent binding thus suggesting the location of the a.a. residue in pos. 639 within the transcription initiation site.

The most widely applied approaches to the study of T7 RNAP are X-ray analysis [1] and the investigations of different mutants or otherwise modified enzymes [2,3]. The above methods gave mainly the information about the enzyme alone but only indirect data regarding its functioning in active complex.

In the present work we used the synthetic promoters with modified CMP residues contaning the additional β-D-ribofuranosyl group in the 2'-position of ribofuranose ring[4]. Periodate oxidation followed by the treatment of sodium borohydride may result in the specific covalent binding of oxidized group with the closely located lysine residues of the enzyme, provided that such proximity really takes place in the native complex structure. As T7 RNAP interacts with the coding chain near the initiation site[5], the modified CMP residues were inserted in positions (+1) or (+2) of the promoter.

T A A T A C G A C T C A C T A T A G
A T T A T G C T G A G T G A T A T C C T G A
+1 +2



in position +1 (duplex I)

in position +2 (duplex II)

The studies of the interactions of modified promoters with T7 RNAP have demonstrated the complete lack of RNA synthesis. On the other hand, all templates investigated inhibited the reaction competing with unmodified templates thus providing the retaining of its affinity to the enzyme.

This fact allowed us to study the possibility of covalent binding of the enzyme with the $NaIO_4$-oxidyzed modified promoters following by $NaBH_4$ reduction. However, according to PAGE analysis, no covalent complexes of the wild type T7 RNAP and ^{32}P-labeled modified promoters were found. As the periodate-oxidized ribose group is highly specific reagent for lysine residues the lack of covalent binding may be due to the absence of such residues in the contact area. The use of partially active T7 RNAP mutant T7 RNAP containing Tyr639Lys substitution demonstrated the formation of the covalent complex of enzyme with promoter modified in (+2) position while the duplex modified in (+1) position did not bind covalently to this mutant. The data obtained suggest that amino acid residue in position 639 is located near the initiation site of the productive T7 RNAP-promoter complex.

REFERENCES

1. Sousa, R.; Chung ,Y.J.; Rose, J.P.; Wang , B.-C. *Nature*, **1993**, *364*, 593-599.

2. Osumi-Davis, P.A.; Sreerama, N.; Volkin, D.B.; Middaugh, R.C.; Woody, R.W.; Woody, A.-Y. M. *J. Mol Biol.*, **1994**, *237*, 5-. 19.

3. Maksimova, T.G.; Mustaev, A.A.; Zaychikov, E.F.; Lyakhov, D.L.; Tunitskaya, V.L.; Akbarov, A. Kh.; Luchin, S.V.; Rechinsky, V.O.; Chernov, B.K.; Kochetkov, S.N. *Eur. J. Biochem.*, **1991**, *195*, 841-847.

4. Mikhailov, S.N.; Efimtseva, E.V.; Gurskaya, G.V; Zavodnik, V.E.; De Bruyn, A.; Rozenski, J.; Herdewijn, P. *J. Carbohydr. Chem.*, **1997**, *16*, 75-92.

5. Muller, D.K.; Martin, C.T.; Coleman J.E. *Biochemistry*, **1989**, *28*, 3306-3313.