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Affinity modification of human chromatin with reactive derivatives of oligonucleotides

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Reaction of 4-(*N*-2-chloroethyl-*N*-methylamino)benzylphosphamides of oligonucleotides (RCl-$(pT)_{16}$ and RCl-$(pApC)_6$) with human chromatin in intact nuclei and with metaphase chromosomes has been investigated. The oligonucleotides were targeted to poly(A) and poly(TG)-repeating DNA sequences. It was found that the reagents alkylate DNA and some proteins due to specific complex formation. The affinity character of the reaction was proved by the fact that free corresponding oligonucleotides taken in excess or preliminary treatment of chromatin with S_1-nuclease both prevent the biopolymers from modification. The results obtained evidence that in human chromatin there are open DNA sequences available for affinity modification with oligonucleotide derivatives. Analysis of patterns of modified proteins within these chromatin areas may give a key to the structure of these chromatin sites.

Human chromatin; Affinity labeling; DNA-binding protein; DNA-repeat; Antisense oligodeoxynucleotide; Hela

1. INTRODUCTION

Oligonucleotide derivatives capable of binding to specific target nucleic acids and affect their functions are becoming an efficient tool of nuclear biologists and they are considered as potential therapeutics exerting their action at the level of genome functioning (for reviews see [1,2]). Recent progress in development of oligonucleotide derivatives targeted to single-stranded nucleic acids has stimulated design of the derivatives for targeting double-stranded DNA [3–6]. It was found that derivatives of pyrimidine and purine oligonucleotides can form specific triple-stranded complexes with corresponding oligopurine–oligopyrimidine stretches in dsDNA. Although such sequences are often found in viral and mammalian DNA, approaches for targeting arbitrary nucleotide sequences in dsDNA remain to be developed. The problem of targeting DNA at any site may be solved if the molecule is locally unwound, exposing single-stranded sequence which can form complementary complexes with oligonucleotides. One could expect that local unwinding of DNA occurs naturally in the course of transcription and replication. In our experiments on affinity modification of DNA in murine chromatin with alkylating derivatives of oligonucleotides we have found that the derivatives indeed can form complementary complexes with some single-stranded regions of the chromatin DNA, although the nature of these regions is perhaps different from those appearing due to replication and transcription [7–11].

In the present paper, we report the results of sequence-specific chemical modification of human chromatin with 4-(*N*-2-chloro-ethyl-*N*-methylamino)benzylphosphamides of oligonucleotides which are targeted to oligo(A) and oligo(TG)-sequences.

Abbreviations: ClR-$(pT)_{16}$, 4-(*N*-2-chloroethyl-*N*-methylamino)benzylphosphamide of $d(pT)_{16}$; ClR-$(pApC)_6$, 4-(*N*-2-chloroethyl-*N*-methylamino)benzylphosphamide of $dp(AC)_6$.

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2. MATERIALS AND METHODS

Chemicals and enzymes were from Sigma and Merck. [γ-^{32}P]ATP of high specific activity (>6000 Ci/mmol) was obtained from the Institute of Nuclear Physics, Uzbek Academy of Sciences. Oligonucleotides and the alkylating derivatives were synthesized according to the described methods [12,13]. Labeling of oligonucleotides at the 5'-end was done with polynucleotide kinase and [γ-^{32}P]ATP [14]. The nuclei from Hela cells and metaphase chromosomes were isolated by the procedure described in [9,15]. Chromatin was incubated with 1×10^{-6} M oligonucleotide derivatives in buffer 1 (buffer 1: 0.34 M sucrose, 4 mM EDTA, 60 mM KCl, 15 mM NaCl, 4 mM $CaCl_2$, 0.15 mM Tris-HCl, pH 7.6) for 20 h at 25°C. DNA was isolated from chromatin by phenol extraction at pH 8 in the presence of 0.5% SDS after treatment of chromatin with ribonuclease A and proteinase K or DNA was separated from proteins by ultracentrifugation according to [10]. Protein's supernatant was dialyzed against 0.01 M Tris-HCl, pH 7.3, 0.1 mM PMSF and incubated with a micrococcal nuclease to remove the oligonucleotide moiety crosslinked to the proteins [10] and was analysed by electrophoresis according to [16]. The extent of DNA modification was calculated after the complete removal of the noncoupled oligonucleotide derivatives from the DNA by gel-chromatography on Sephadex G-100 under dissociating conditions (7 M urea).

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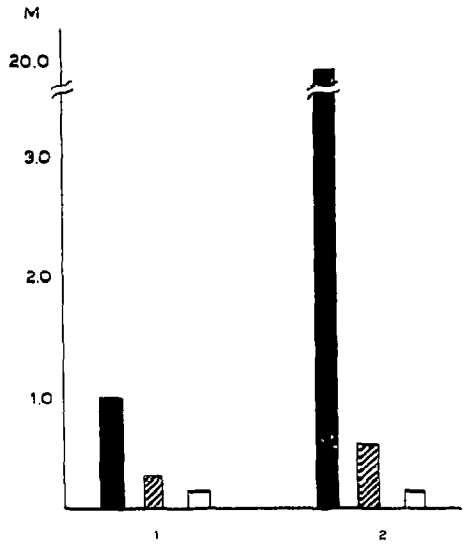

Fig. 1. Relative extent of DNA modification in Hela nuclei with RCl-[^{32}P]-$(pT)_{16}$ (1) and RCl-[^{32}P]-$(pApC)_6$ (2). M = modification extent, moles of reagent per 10^6 moles of DNA nucleotides. (■) Modification without addition of free oligonucleotides; (▨) modification in the presence of 10-fold excess of free oligonucleotide; (□) modification after preliminary treatment of nuclei by S_1-nuclease.

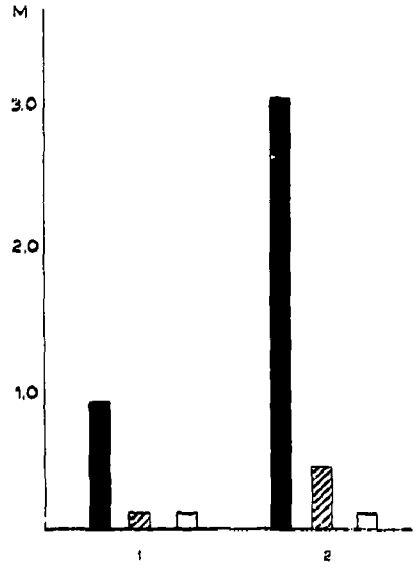

Fig. 2. Relative extent of DNA modification in metaphase chromosome with RCl-[^{32}P]-$(pT)_{16}$ (1) and RCl-[^{32}P]-$(pApC)_6$ (2). M = modification extent, moles of reagent per 10^6 moles of DNA nucleotides. (■) Modification without addition of free oligonucleotides; (▨) modification in the presence of 10-fold excess of free oligonucleotide; (□) modification after preliminary treatment of chromosome by S_1-nuclease.

3. RESULTS AND DISCUSSION

Since the nuclear membrane is permeable for alkylating derivatives of oligonucleotides [10], in this study intact Hela nuclei were treated with the reagents RCl-[^{32}P]-$(pdT)_{16}$ and RCl-[^{32}P]-$(pdApdC)_6$. These oligonucleotides are targeted to the oligo(A)- and oligo(TG)-repeats of DNA which are widely distributed in eukaryotic DNA.

Fig. 1 shows the results of the experiment. DNA of the Hela interphase nuclei is substantially more available for modification with the RCl-[^{32}P]-$(pdApdC)_6$ than with the RCl-[^{32}P]-$(pdT)_{16}$. The excess of the cold oligonucleotide of a same sequence, as the reagent, considerably decreases the extent of modification, thus suggesting sequence-specific processes in both cases. It should be emphasized that deproteinated DNA from the same cells does not react with the oligonucleotide derivatives, which means that the chromatin DNA should have some structural features allowing oligonucleotide binding; it can rather be attributed probably to the existence of the strongly negatively supercoiled DNA regions (open single-stranded sequences or regions where DNA can unwind in the presence of oligonucleotides).

It was shown that the extent of DNA modification was not influenced by the additional treatment with ribonuclease under dissociating conditions (7 M urea). Therefore the oligonucleotide binding cannot be attributed to the interaction with RNA present in the chromatin preparation.

Mild preliminary treatment of the nuclei with S_1-nuclease, which digests single-stranded parts of chromatin DNA, prevents the DNA from modification with oligonucleotide derivatives. This suggests that in both cases the reaction proceeds with single-stranded DNA sequences via formation of the double-stranded complexes. Fig. 2 demonstrates that specific chemical modification of DNA in metaphase chromosome can also occur although less efficiently as compared to the interphase nuclei, particularly for the $(pApC)_6$-derivative.

It was found that ClR-$pT)_{16}$ and ClR-$(pApC)_6$ react also with chromatin proteins (Fig. 3). From the data shown in Fig. 3 it is seen that the two reagents label different sets of proteins. Nine proteins are labeled with ClR-$(pT)_{16}$ and 14 with ClR-$(pApC)_6$ in intact interphase Hela nuclei. Four proteins from Hela nuclei treated with RCl-$(pT)_{16}$ are alkylated specifically (molecular masses about 19, 47, 61, >66 kDa), since their modification was inhibited both by excess of the cold $(pT)_{16}$ and by preliminary S_1-nuclease treatment of nuclei (Fig. 3). A more complex set of modified proteins was obtained by treatment of Hela nuclei with RCl-$(pApC)_6$ among the 14 modified proteins at least 7 (with molecular masses near 19.5, 21, 25.5, 31, 33, 43 and >66 kDa) were alkylated specifically. These proteins were protected from the reagent by the excess of free $(pApC)_6$ and the reaction required the presence of single-stranded DNA parts. These results suggest that these specifically modified proteins are located near the corre-

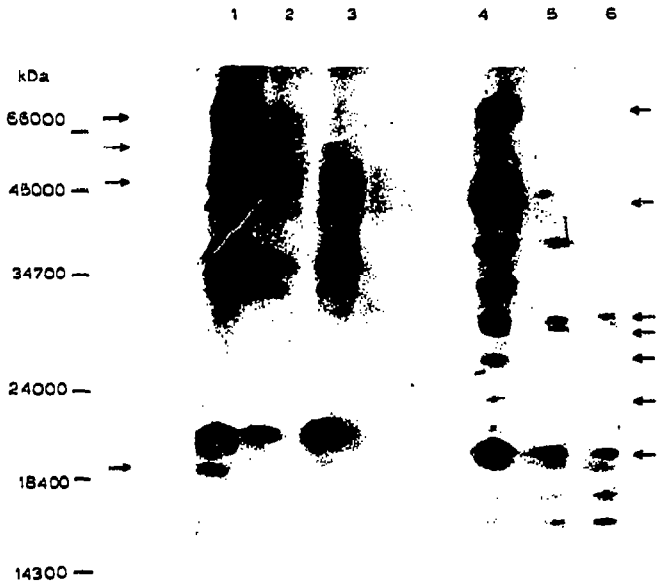

Fig. 3. Analysis of modified chromatin proteins by SDS-electrophoresis. (Lane 1) Modification with RCl-[^{32}P]-$(pT)_{16}$; (lane 2) modification with RCl-[^{32}P]-$(pT)_{16}$ in the presence of 10-fold excess of free $(pdT)_{16}$; (lane 3) modification with RCl-[^{32}P]-$(pT)_{16}$ after preliminary treatment by S_1-nuclease; (lane 4) modification with RCl-[^{32}P]-$(pApC)_6$; (lane 5) modification with RCl-[^{32}P]-$(pApC)_6$ in the presence of excess of free $(pApC)_6$; (lane 6) modification with RCl-[^{32}P]-$(pApC)_6$ after preliminary treatment by S_1-nuclease.

sponding target nucleotide sequences which are available for the complex formation.

The results obtained provide evidence that there are open DNA sequences in human chromatin which can be affinity-modified by oligonucleotide derivatives. Analysis of modified proteins within these chromatin areas may give a key to the structure of these chromatin sites.

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