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Chromatin proteins surrounding the d(G-T)$_n$ repeats and polyamine influence as revealed by photoaffinity labeling with reactive pd(A-C)$_6$ derivatives

L.N. Bozhenok, E.B. Khazina, E.L. Chernolovskaya*, N.D. Kobets

Institute of Bioorganic Chemistry, Siberian Division of the Russian Academy of Sciences, 8, Lavrentiev ave., Novosibirsk 630090, Russia

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Abstract The complementary-addressed modification of DNA and proteins in chromatin using photoreactive derivatives of pd(AC)6 has been studied. These oligonucleotides form complementary complexes with specific DNA sequences and modify both DNA and proteins in the vicinity of these regions, and can be used for investigation of the protein environment in DNA. We have demonstrated that photoreactive derivatives of oligonucleotides can quickly and efficiently modify chromatin proteins and seem to be promising for investigation of perturbations in chromatin structure during the cell cycle. A comparison between modified chromatin from synchronized cells has demonstrated differences in the sets of proteins modified in the S and G_1/S phases of the cell cycle. An increase in spermine and spermidine concentrations leads to an increase in modification of definite chromatin proteins. It can be supposed that the B-Z transition that can be stabilized by the presence of natural polyamines is one of the reasons for the presence of single-stranded DNA regions, containing sets of (dG-dT)$_n$ and accessible for interaction with complementary oligonucleotides.

Key words: Chromatin; Protein; Oligonucleotide derivative; Photoaffinity labeling

1. Introduction

Chromatin is a most complicated supramolecular structure of an eukaryotic cell responsible for storage, repair, multiplication and expression of genetic information. The study of the structure and mechanism of functioning of chromatin at the molecular level is one of the most intriguing problems in modern molecular biology. Two main complications pose the problem. The first one is the extreme complexity of the chromatin structure due both to the large number of components and to the extraordinarily complicated spatial structure of the system, in which a huge number of proteins form contacts with DNA. The other one is that the chromatin structure is significantly affected by both changes of the phases of the cell cycle and changes in the surrounding medium.

To investigate the arrangement of specific sites in the chromatin structure we proposed affinity modification with reactive derivatives of oligonucleotides targeted to specific nucleotide sequences.

The first experiments of this type were performed with *N*-2-chloroethyl-*N*-methylaminobenzyl derivatives of oligonucleotides. It was demonstrated that the compounds react with DNA in conditions where double-stranded DNA (dsDNA) devoid of chromatin proteins does not interact with these reagents [1–4]. Thus it was concluded that chromatin DNA possesses some peculiar features favoring interaction with reactive oligonucleotide derivatives. It was also observed that modification of DNA is accompanied by labeling of a number of proteins. The modification observed was sequence-specific and correlated with sensitivity of the same chromatin regions to S1 nuclease treatment. It was shown that the specific set of modified chromatin proteins depends both upon the sequence of oligonucleotide and upon the functional state of chromatin.

In the present paper, we report the results of sequence-specific chemical modification of human chromatin with arylazido derivatives of dodecadeoxyribonucleotide pd(AC)$_6$ targeted to (G-T)$_n$ repeats of DNA. The use of photoreactive groups opens up the possibility of performing rapid labeling in the course of rearrangements accompanying transition processes [5]. The (G-T)$_n$ repeats are abundant in eukaryotic DNA (10^5 copies of d(GT)$_n$ with $n > 25$ in the human genome) [6,7]. It was shown that d(G-T)$_n$ sequences capable of forming left-handed Z-DNA are localized at the sites of a facile B-DNA to Z-DNA transition [8,9]. Recent mapping of such sequences with the potential of forming Z-DNA in human genes revealed a non-random distribution, with a strong bias towards location near the sites of transcription initiation [10]. Potentially Z-DNA-forming alternating purine-pyrimidine sequences are widely dispersed in native DNAs, including the enhancer elements of several genes [6,11], suggestive of a functional role for Z-DNA in gene expression.

2. Materials and methods

Chemicals and enzymes were from Sigma and Merck. [γ-^{32}P]ATP of high specific activity (>6000 Ci/mmol) was prepared by D. Semenov (Novosibirsk Institute of Bioorganic Chemistry). Oligonucleotides and the photoreactive derivatives were synthesized according to the described methods [12–14]. Azidoaniline derivatives of pd(AC)$_6$ were kindly provided by T.S. Godovikova. Nuclei from HeLa cells were isolated by the procedure described in [4]. All procedures were carried out at 4°C and all buffers were supplemented with 0.1 mM phenylmethylsulfonyl fluoride (PMSF). Chromatin was incubated with 1×10^{-6} M oligonucleotide derivatives in buffer 1 (0.15 mM Tris-HCl, pH 7.6, 0.34 M sucrose, 4 mM EDTA, 60 mM KCl, 15 mM NaCl, 4 mM $CaCl_2$, 0.5 mM spermidine, 0.15 mM spermine) or in buffer 2 (1 mM sodium cacodylate, pH 7.4, 0.15 mM EDTA, 50 mM NaCl), according to the procedure described in [14]. Modified chromatin proteins were analyzed by SDS-gel electrophoresis according to [3]. In some of the control experiments preliminary S1 nuclease treatment of nuclei was used as described in [4].

3. Results and discussion

Photoreactive derivatives of pd(AC)$_6$, bearing 2-nitro-5-azi-

*Corresponing author. Fax: (7) (383) 2-33-36-77.
E-mail: elena_ch@niboch.nsc.ru

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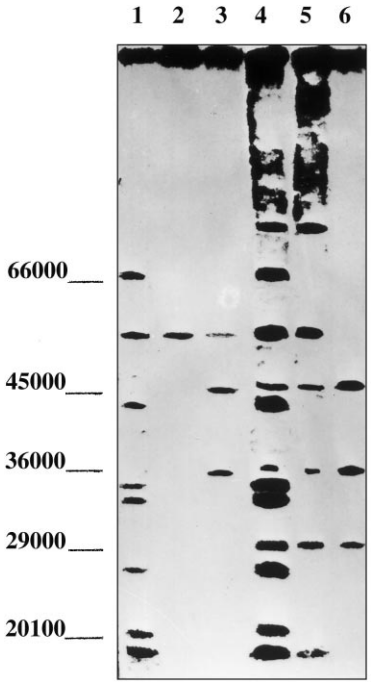

Fig. 1. SDS-PAGE of modified proteins after affinity labeling with photoreactive derivatives of pd(AC)$_6$ in HeLa nuclei. Lanes 1–3: with reagent I; lanes 4–6: with reagent IIb (lanes 2 and 5: modification in the presence of excess of free pd(AC)$_6$; lanes 3 and 6: modification after preliminary treatment with S1 nuclease).

dobenzoyl or *p*-azidoaniline residues tethered to the 5′-phosphate of the pd(AC)$_6$ end via a phosphoamide bond (2-NO_2,5-N_3-C_6H_4-C(O)NH-$(CH_2)_3$NH-pd(AC)$_6$ (reagent I) or $N_3C_6H_4$NH-$(CH_2)_n$-NH-pd(AC)$_6$ (n=2, 4 or 6) (reagents IIa–c) were used to study proteins surrounding $(G\text{-}T)_n$ repeat DNA in human chromatin which are located in sites with some structural features allowing oligonucleotide binding. Chromatin isolated from HeLa cells was incubated with 10^{-6} M oligonucleotide derivatives for 30 min at room temperature and then irradiated at wavelengths of 303–365 nm. The modified proteins were isolated and analyzed by SDS-gel electrophoresis. Fig. 1 shows the patterns of modified proteins in HeLa chromatin incubated and irradiated with reagent I (lanes 1–3) and with reagent IIb (lanes 4–6). It can be seen that reagent IIb provides a higher modification efficiency as compared with reagent I. Both reagents label a similar set of nuclei proteins (with molecular masses near 19.5, 21, 25.5, 31, 33, 43, and >66 kDa). The proteins were modified specifically, since their modification was inhibited by an excess of pd(AC)$_6$ (see Fig. 1, lanes 2 and 4). Mild preliminary treatment of the nuclei with S1 nuclease, which digests single-stranded parts of chromatin DNA, also prevented proteins from being modified (lanes 3 and 6). This suggests that the reaction occurs with proteins in the vicinity of single-stranded DNA sequences, where oligonucleotide conjugates form double-stranded complexes.

To find the optimal length of the spacer connecting the photoreactive group with the addressing oligonucleotide moiety we tested *p*-azidoaniline derivatives IIa–c. It was shown that reagent IIb with the $(CH_2)_4$ spacer is most efficient in modifying nuclear proteins (data not presented).

Taking into account the dynamic structure of chromatin we tested our approach for the investigation of structural transitions of the chromatin during the cell cycle. Fig. 2 demonstrates the results of the modification of HeLa nuclei with reagent IIb (n=4), isolated from cells, synchronized at the border of G_1 and S phases (in G_1/S) and in S phase. HeLa cells were synchronized at the border of G_1 and S phases using double thymidine blockade. Subsequent 3 h incubation of the synchronized cell culture after removal of the inhibitor results in a cell culture synchronized in the middle of S phase, when DNA replication achieves its maximum. It is seen that the patterns of modified proteins are somewhat different in G_1/S and S phase nuclei: proteins with molecular masses about 50 and 83 kDa are modified in the nuclei from G_1/S HeLa cells and these proteins are not labeled in the chromatin from S phase HeLa cells. In the same time the modification of the protein with molecular mass about 68 kDa occurs only in the nuclei from S phase cells (Fig. 2: dots indicate the most pronounced differences in patterns of the labeled proteins). The above results suggest that affinity labeling with reactive derivatives of oligonucleotides might be applied for examination of different features of chromatin structure and for investigation of the protein environment of specific unwound DNA sequences.

To gain insight into the nature of the chromatin structures susceptible to modification, we investigated the effect of polyamines on the modification of chromatin. It was shown that $(G\text{-}T)_n$ sequences capable of forming left-handed Z-DNA are localized at the sites of a facile B-DNA to Z-DNA transition [8,9]. The B-DNA to Z-DNA transition can provoke a local unwinding of DNA at these sites exposing single-stranded sequences available for interaction with complementary oligonucleotides. It was demonstrated that the induction and stabilization of a left-handed Z-DNA conformation under phys-

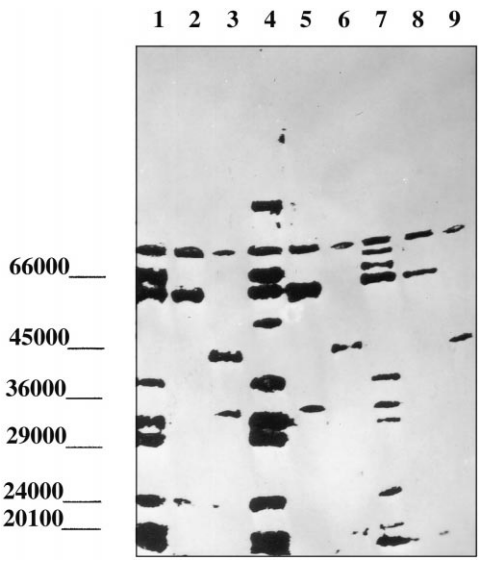

Fig. 2. SDS-PAGE of modified proteins after affinity labeling with reagent IIb in HeLa nuclei isolated from cells. Lanes 1–3: from unsynchronized cells; lanes 4–6: synchronized at the border of G_1 and S phases; lanes 7–9: synchronized in S phase (lanes 2 and 5: modification in the presence of excess of free pd(AC)$_6$; lanes 3 and 6: modification after preliminary treatment with S1 nuclease). Dots indicate the most pronounced differences in patterns of labeled proteins.

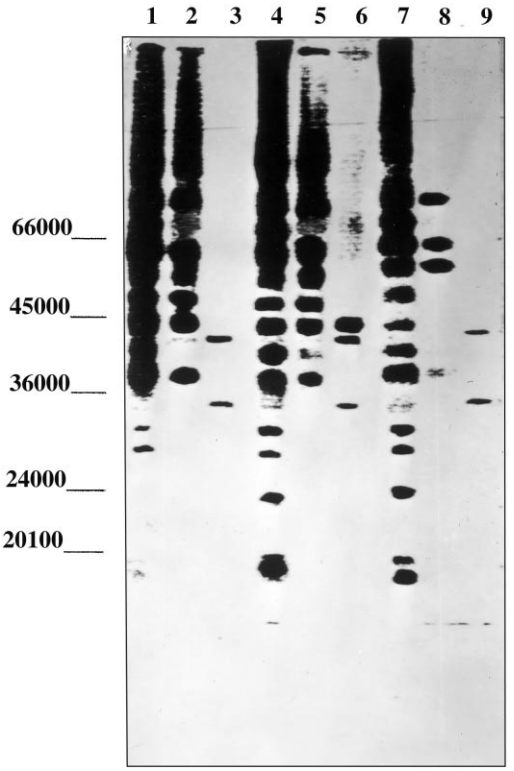

Fig. 3. SDS-PAGE of modified proteins in HeLa nuclei in buffer without polyamines (lanes 1–3) and in the same buffer with 2.8 mM spermidine (lanes 4–6) and with 0.5 mM spermine (lanes 7–9). Lanes 2, 5 and 8: modification in the presence of an excess of free $pd(AC)_6$; lanes 3, 6 and 9: modification after preliminary treatment with S1 nuclease).

iologically relevant ionic conditions is provoked by the interaction of natural polyamines (spermidine and spermine) with specific DNA sequences [15–17]. We found that the chromatin modification under standard modification conditions (see Section 2) is affected by the spermine and spermidine concentrations (data not presented) and the maximal reaction yield is achieved at 2.8 mM spermidine or 0.5 mM spermine. We compared the protein modification in the isolated nuclei in buffer without polyamines (buffer II) and in the same buffer with 2.8 mM spermidine (buffer IIa) and with 0.5 mM spermine (buffer IIb). These results are shown in Fig. 3. Photomodification experiments in the presence of 2.8 mM spermidine (lanes 4–6) and 0.5 mM spermine (lanes 7–9) revealed a higher extent of modification for a number of proteins as compared with photomodification of chromatin in the absence of polyamines (in buffer II). It can be seen that the most pronounced dependence of protein modification on the natural polyamines is observed for chromatin proteins with molecular masses 19.5, 21 and 25.5 kDa. It seems likely that these chromatin proteins are associated with the corresponding target $d(G\text{-}T)_n$ repeats of DNA which become available for complex formation with the reagent due to $B \rightarrow Z$ transition of DNA creating open single-stranded regions.

Based on chemical modification of the DNA stretches located in the areas of its local unwinding, our approach may be a useful tool for elucidating chromosome structure. Analysis of modified proteins within these chromatin areas may give a key to the structure of these chromatin sites.

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