Significance of Specific Protein S-S Bonds in the Structural-Functional Organization of DNA

V. A. Struchkov and N. B. Strazhevskaya

UDC 577.112.386

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 118, № 8, pp. 180-183, August, 1994
Original article submitted December 2, 1993

The role of S-S bridges of residual protein in the structural organization of DNA is investigated. The effects of various S-S splitting agents on the naturally occurring DNA-RP protein complexes isolated from various eukaryotic and prokaryotic cells are studied. It is demonstrated that, depending on the incubation conditions, thiols induce dissociation of the DNA-RP complexes to double-strand fragment-DNA subunits of varied size. It is found that the DNA-RP complexes contain specific S-S bonds that may determine different levels of DNA organization in the chromosome.

Key Words: *thiols; protein S-S bridges; DNA structure*

Investigation of the residual protein (RP) that remains firmly bound to DNA irrespective of isolation technique and produces DNA-polypeptide complexes is important for a better understanding of the structural-functional organization of DNA in the eukaryotic chromosome [18,21,22]. These DNA-polypeptide complexes display site-specific localization in the eukaryotic genome [22]. Of particular importance in this context is the evidence that S-S bridges of RP covalently bound to DNA are involved in the tandem organization of the DNA subunits in the chromosome [14,15,17] and in the attachment of the replicon loops to the nuclear matrix [3,16], i.e., in a higher level of DNA organization.

This paper summarizes the results of our previous studies [5,6,9,19] on pH-dependent thiol-induced fragmentation of naturally occurring eukaryotic and prokaryotic DNA-RP complexes, on the determination of the size of thiol-induced DNA subunits and their secondary structure, and on the effects of thiols on the RP composition in these complexes.

Oncological Research Center, Russian Academy of Medical Sciences, Moscow. (Presented by N. N. Trapeznikov, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Two DNA fractions - a water-soluble supramolecular DNA complex (SM DNA) and water-insoluble DNA of the phenol nuclear matrix (PNM DNA) - were isolated by the phenol method from eukaryotic cells (rat liver and thymus, loach sperm cells and erythrocytes; hen erythrocytes, P 388 leukemia cell line) and T4 phage. The isolation procedures for SM DNA and PNM DNA, the conditions for incubation with mercaptoethanol (ME), dithiothreitol (DTT), glutathione reductase, and sodium borohydride, as well as electrophoresis of proteins, elastoviscosimetry, sedimentation, and melting of DNA were described in detail elsewhere [5-7].

RESULTS

SM DNA contains 1-3% immunogenic RP consisting 30-50% of acid amino acids (predominance of glutamic acid) and actively incorporating ^{35}S-methionine [8]. ELectrophoresis [7] showed that eukaryotic SM DNA is characterized by the presence of a protein quartet in the 50-70 kD region and by the presence of several polypeptides with a molecular weight of 20-40 kD. The protein quartet is resistant to RNAase A, phospholipase C,

0007-4888/94/0008-0875$12.50

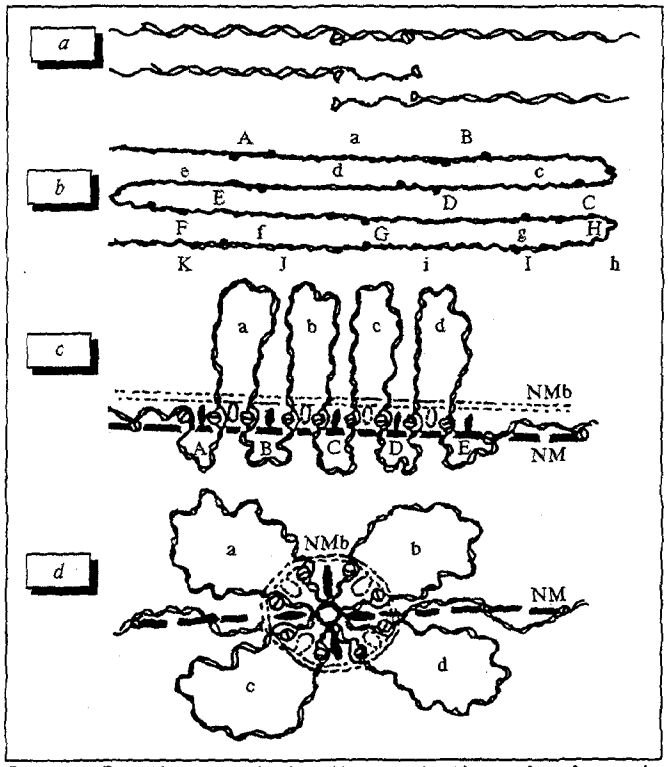

Fig. 1. Putative quasisubunit organization of eukaryotic chromosomal DNA. *a*) tandem joining of DNA subunits with the aid of peptide S–S bonds localized in antiparallel DNA strands at a distance of 196 bp; *b*) thiol–induced skewed double–strand rupture with the formation of sticky ends; c) quasisubunit organization of DNA with peptide S–S bonds and complementary sticky ends; *d*) loop organization of DNA (domains) by means of blocked polypeptides: *A–E*) small loops, *a–e*) large loops; *NM*: nuclear matrix; *NMb*: nuclear membrane; black circles: topoisomerase II; white circles: lipids; *D*: chromomeric organization of DNA.

pancreatic lipase, proteinase K, and 0.2% ME at pH 7.2. This protein group is similar in molecular weight and resistance to proteinase K to the proteins described in other studies [14,18,21,22], which are believed to confirm the superhelical organization of DNA [14].

Eukaryotic PNM DNA proteins (5-10% protein of DNA) are represented by any more fractions: 15-30 bands in the 14-120 kD region, including the 50-70 kD protein quartet [9], implying that these proteins are involved in the DNA contact with the nuclear skeletal structures. Incubation of PNM DNA preparations with 0.2% ME induces dissociation primarily of proteins with a molecular weight higher than 50 kD, the effect being more pronounced at pH 5.0 than at pH 7.0 [9].

A pH-dependent (at acid and neutral pH) thiol-induced fragmentation of these DNA complexes to different-size subunits was established when the effects of various S-S splitting agents (ME, DTT, sodium borohydride, and glutathione reductase) were studied; this indicates the presence of several specific protein disulfide bonds in chromosomal DNA and their general biological significance [5,6,19]. We think that thiol-induced fragmentation of SM DNA and PNM DNA results from the reducing influence of thiols on the RP S-S groups covalently bound to DNA and participating in the quasisubunit organization of DNA. As already reported [6], degradation of DNA complexes in the presence of thiols due to the presence of nucleases is unlikely. A possible contribution of apurinization to this phenomenon cannot be excluded; however, it is difficult to explain ME-induced fragmentation of DNA by apurinization based on the available data on the rate of this reaction at 37°C [1]. On the whole, our results are consistent with those of others [14-17] demonstrating an important role of S-S bridges of RP in the organization of chromosomal DNA. However, in contrast to the above studies, we tested several types of disulfides in the DNA complexes. This can be explained by the fact that the reactive ability of an S-S bond in a protein depends on a number of factors: the accessibility of this bond, the nature of the neighbouring groups, and the intensity of noncovalent interactions near S-S groups [12].

Type I disulfides were found in SM DNA of eukaryotes and T4 phage. This type is resistant to proteolytic enzymes and was reduced by ME only at an acid pH (4.4-5.9); prolonged (5-10 days) incubation with thiols resulted in complete degradation to subunit fragments with a molecular weight of 5×10^5 D [5,6,10,19] comparable in size to genes. Disulfides sensitive to ME at acid pH values were also tested in eukaryotic PNM DNA [5]. Subunits of a similar size ($5\text{-}7\times10^5$ D) were obtained after a 2-week incubation with 0.05 M EDTA (pH 7.0, 0°C) of DNA isolated from thymic nuclei using pronase-sarcosyl-3 M NaCl [20]. These researchers believe that the DNA degradation is due to the fact that metalloproteins (0.9-1.4 kD) that provide for the subunit organization of DNA in eukaryotic chromatin are embedded in the sugar-phosphate backbone of DNA. The presence of various metals in chromatin, DNA, and nonhistone proteins has been demonstrated [16,20]. Single-strand DNA subunits (8.5×10^6 D) were found in DNA preparations treated with pronase and alkali, as well as double-strand DNA subunits (16.1×10^6 D) after treatment with pronase and SI-nuclease [21,22]. These findings indicate that 1% of pronase-resistant serum-containing protein is covalently bound to the 3' and 5' ends of DNA in antiparallel strands by phosphodiester bonds via O^4-phosphotyrosine.

Type II disulfide bridges were found in only eukaryotic DNA (this type was absent from the DNA of phage T4) with the use of glutathione reductase, indicating the presence of mixed disulfides in the protein. The subunit size was $71\pm1з10^6$ D [5].

Type III disulfides were found in eukaryotic PNM DNA with the use of ME at a neutral pH [5]. They probably control the domain-cluster DNA level (10^9 D). A similar type of fragmentation was demonstrated upon incubation of neutral lysates of animal cells with ME and pronase [14,17]. The important role of disulfides in the nuclear matrix organization has been extensively discussed in the literature [3].

Type IV disulfides (probably metal-disulfides) were found in eukaryotic SM DNA upon incubation with DTT in the presence of EDTA at pH 8.0; after prolonged incubation (5-10 days) the complex dissociated to $18.6\pm1.0\times10^6$ D subunits [5,6] which are comparable in size to a transcripton.

Type V disulfides (probably metal-disulfides) were found in eukaryotic SM DNA upon prolonged incubation with ME in the presence of EDTA at pH 8.0. There was no DNA fragmentation under these conditions; however, the elastoviscosity of the complexes is completely destroyed, indicating the implication of these S-S bridges in the organization of the replicon loops (of $192\pm5\times10^6$ D) [5,6]. Thus, several types of specific S-S bonds are present in eukaryotic DNA complexes with different degrees of complexity and in DNA of phage T4; reduction of these bonds with thiols (ME and DTT) leads to the formation of different double-strand DNA subunits comparable in size to a gene, transcripton, replicon, and domain, the formation being dependent on the pH, the presence of EDTA, and the incubation period. This suggests that a multicomponent subunit structure of DNA is a common phenomenon for eukaryotic and prokaryotic cells; the size of DNA subunits may vary from 5×10^5 D [5,6,10,19,20] to 10^9 D [14,17].

There is no consensus regarding the mechanism of thiol-induced fragmentation of chromosomal DNA. According to the model [15], the S-S bridges of DNA-bound RP that join DNA fragments in tandem fashion coincide with the preexisting single-strand ruptures in antiparallel chains of an intact molecule, and, therefore, a double-strand rupture of DNA occurs when S-S groups are reduced by thiols. However, other researchers [14] have shown that thiols can induce only single-strand ruptures, which causes twisted DNA forms to become circular. From an analysis of our data on heat denaturation of ME-induced DNA fragments at acid pH it can be concluded that there are sticky ends, and on the basis of this conclusion we can assume that thiol-induced fragmentation of eukaryotic DNA is a result of skewed ruptures in single-strand breaks in antiparallel strands lying close to one other [6,19]. It was calculated that at least 196 ± 12 base pairs are involved in such a rupture. This value is characteristic of SM DNA isolated from different eukaryotic cells and has been reliably tested for $0.5\text{-}2.0\times10^6$ D subunits.

Thus, not only S-S bridges of the DNA-bound RP but also the complementary sticky ends of its subunits may be implicated in the tandem quasisubunit organization of chromosomal DNA (Fig. 1). It is hypothesized that the tandem of two DNA subunits is achieved via the S-S bridges of 4 polypeptides located in pairs in antiparallel strands at a distance of 196 bp. Assuming that SM DNA contains an average of 1% RP with mean molecular weight 60 kD, 2-3 kD polypeptides are involved in the tandem arrangement of $0.5\text{-}2.0\times10^6$ D DNA subunits. However, the molecular weight of DNA-bound RP is much greater: 12-70 kD [7,9]. Therefore, we suggested that these calculated low-molecular-weight polypeptides should be arranged in larger peptide blocks via weak peptide bonds, and these peptide blocks were identified by us [7,9] and others [14,16-18,21,22] on electrophoregrams. This is a reasonable assumption, since DNA-bound polypeptides can degrade to smaller units (18 and 36 kD) under the conditions of acid hydrolysis (10% TCA, 12 h, 37°C) as was demonstrated in our studies. Moreover, smaller polypeptides (2-3 kD) remain bound to DNA after a more stringent hydrolysis (6 M HCl, 90°C, 1.5 h) [18].

We believe that blocked polypeptides can serve as a basis for the formation of small and large DNA loops (Fig. 1). Small loops, the regions of sticky ends of quasisubunits, can be the sites interacting with DNA topoisomerase II [3,4] and specific DNA-bound lipids [11] that are localized at the base of the DNA loop on the nuclear matrix and take part in replication and transcription. A possible joint site-localization of topoisomerase II and lipids in the DNA loop base is supported, on the one hand, by the presence of 50 and 63 kD proteins in SM DNA isolated from rat thymus and liver [7] comparable in size to the topo II subunits (51 and 60 kD) [3,4] and, on the other, by fragmentation of SM DNA to $60\text{-}10^6$ D subunits under the influence of lipase and phospholipase C [11]. The large loops are carriers of genetic information. S-S bridges can serve as flanks between genes involved in the exchange of DNA fragments. After histonization these loops can serve

as a basis for cohelixation of DNA strands in superstructures: nucleosomes, nucleomeres, and chromomeres. In this case blocked and nonblocked DNA-bound polypeptides may represent chromomeric and nonchromomeric regions of a chromatid, respectively.

It is known that the energy of an S-S bond is only 66 kkal [12]. Consequently, thiol-disulfide bonds in chromatin are a labile dynamic system at which level the oxidation-reduction processes necessary for active cell metabolism readily proceed. A typical example is the participation of thiol-disulfide exchange in the formation of the mitotic apparatus [2] as a whole, and, specifically, in the redox regulation of the DNA-binding activity of fos and jun oncoproteins [13]. Numerous radiobiological studies confirm the functional significance of disulfide bonds in chromatin. Like thiols, ionizing radiation decondenses chromatin and stimulates reparative DNA synthesis [2]. At the same time, it is known that various serum-containing protective agents (for example, aminoethylisothiouronium of SM DNA *in vivo* [8]) are highly effective protectors against radiation [2]. From the above-mentioned findings we concluded that specific protein disulfides may play a hierarchic role in the structural-functional organization of DNA by determining its gene, transcripton, replicon, and domain-cluster levels in the genomes of various biological systems.

REFERENCES

1. M. M. Vilenchik, *Usp. Sovr. Biol.*, **82**, № 3 (6), 371-386 (1976).
2. E. Ya. Graevskii, *Sulfhydryl Groups and Radiosensitivity* [in Russian], Moscow (1969), p. 144.
3. I. B. Zbarskii and S. V. Kuz'mina, in: *Skeletal Structures of the Cell Nucleus* [in Russian], Moscow (1991), p. 242.
4. K. A. Kafiani and I. B. Bronshtein, *Usp. Biol. Khimii*, Moscow (1988), Vol. 29, pp. 84-112.
5. V. A. Struchkov and N. B. Strazhevskaya, *Dokl. Akad. Nauk SSSR*, **307**, № 3, 755-758 (1989).
6. V. A. Struchkov, N. B. Strazhevskaya, and D. Yu. Blohkin, *Byull. Eksp. Biol. Med.*, **113**, № 5, 529-531 (1992).
7. N. B. Strazhevskaya, V. A. Struchkov, and D. Yu. Blokhin, *Radiobiologiya*, **29**, № 4, 441-444 (1989).
8. N. B. Strazhevskaya and V. A. Struchkov, *Ibid.*, **17**, № 2, 163-167 (1977).
9. V. A. Struchkov, D. Yu. Blokhin, and N. B. Strazhevskaya, *Ninth All-Union Symposium "Structure and Function of the Cell Nucleus"* [in Russian], Chernogolovka (1987), pp. 130, 190.
10. V. A. Struchkov, D. Yu. Blokhin, and N. B. Strazhevskaya, *Tenth All-Union Symposium "Structures and Function of Cell Nucleus"* [in Russian], Grodno (1990), p. 69.
11. V. A. Struchkov and N. B. Strazhevskaya, *Biokhimiya*, **58**, № 8, 1154-1175 (1993).
12. Yu. M. Torchinskii, *Sulfhydryl and Disulfide Groups of Proteins* [in Russian], Moscow (1971).
13. C. Abate, L. Patel, F. J. Rauscher, *et al.*, *Science*, **249**, № 4973, 1157-1161 (1990).
14. T. Andoch and T. Ide, *Exp. Cell. Res.*, **74**, № 2, 525-531 (1972).
15. A. L. Dounce, S. SK. Chandra, and P. L. Townes, *J. Theor. Biol.*, **42**, № 2, 275-285 (1973).
16. P. Jeppesen and H. Morton, *J. Cell Sci.*, **73**, № 2, 245-260 (1985).
17. C. S. Lange, *FEBS Lett.*, **44**, № 2, 153-165 (1974).
18. B. Neuer, U. Plagens, and D. Werner, *J. Molec. Biol.*, **164**, № 2, 213-235 (1983).
19. V. A. Struchkov, N. B. Strazhevskaya, and D. Yu. Blokhin, in: *Nuclear Structure and Function*, Eds. J. R. Harris and I. B. Zbarsky (Eds.), New York (1990), pp. 57-60.
20. R. S. Welsh and K. Vyska, *Biochim. Biophys. Acta*, **655**, № 3, 291-306 (1981).
21. D. Werner, W. Krauth, and H. V. Hershey, *Ibid.*, **608**, № 2, 243-258 (1980).
22. D. Werner and B. Neuer-Nitsche, *Nucl. Acid Res.*, **17**, № 15, 6005-6015 (1989).