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THIOL-INDUCED FRAGMENTATION OF CHROMOSOMAL DNA

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The role of S–S groups of residual protein in the tandem organization of subunits of chromosomal DNA has been demonstrated in [6-8]. We have shown [3, 4] that in supramolecular DNA–residual protein complexes, of different complexity, isolated by a mild phenolic method [1], three types of specific S–S bonds are present (mercaptoethanol-dependent at acid and neutral pH, glutathione reductase-dependent), and that these can determine different levels of organization of the DNA not only of eukaryotes, but also of prokaryotes.

In the investigation described below the sedimentation method was used to assess the size of thiol-induced DNA subunits, and their secondary structure was studied by the thermal denaturation method.

EXPERIMENTAL METHOD

Supramolecular DNA complexes, isolated by methods in [1] from loach sperm and erythrocytes, and from hens' erythrocytes, contain 0.15, 0.2, and 0.5% of residual protein respectively; the composition of the protein includes cysteine and 40% of acid amino acids; it consists of 4-5 peptides with mol. wt. of 12-70 kD, of which the duplex of 50-70 kD proteins is constant for different objects [5]. As S–S-cleaving agents we used 2-mercaptoethanol (ME, from "Serva," Germany), dithiothreitol (DTH, from "Serva," Germany), and sodium borhydride (25 mM). The DNA preparations were incubated with the thiols under sterile conditions with the addition of 10 mg% of sodium azide, using the following solutions: 0.07 M NaCl, 0.07 M acetate buffer, pH 4.4-5.9; 0.07 M NaCl, 0.07 M phosphate buffer, pH 8.0; 0.14 M NaCl, 0.025 M EDTA, 0.003 M phosphate buffer, pH 8.0. After treatment with the thiols the DNA samples were dialyzed against 0.14 M NaCl-0.01 M SSC, pH 7.0. W-sedimentograms were recorded by means of a chromatographic densitometer (type KhD, USSR). The sedimentation constant and molecular weight of the DNA were estimated by the method in [10], with correction for the experimental conditions used. DNA from phage T4 and phage lambda, isolated by the method in [1], served as the standard. Floating the DNA (20 μg/ml in solution of 0.14 M NaCl, 0.01 M SSC, pH 7.0) was carried out on an SF-16 spectrophotometer at 260 nm, with exposure of 5 min at the points for reading optical density (interval 5°C).

EXPERIMENTAL RESULTS

As we showed previously by a viscosimetric method [3, 12], a characteristic feature of the thiols (ME and DTH) is fragmentation of supramolecular DNA complexes only at acid pH values (4.1-5.5); the intensity of the effect, moreover, was determined by the incubation time (2-24 h), the nature of the thiol, and the nature of the complex. In

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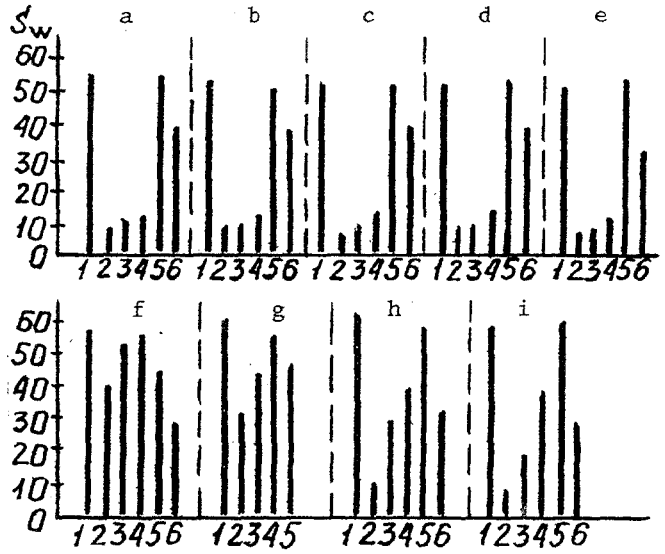

Fig. 1

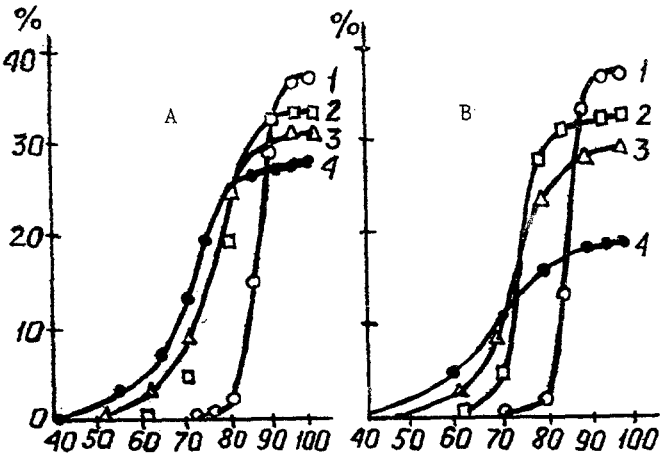

Fig. 2

Fig. 1. Sedimentati[illegible] (S_w) of supramolelcular DNA complex and its thiol-induced subunits (2 μg DNA in 0.14 M NaCl – 0.01 M SSC, pH 7, 5-20% neutral sucrose gradient, L5-75 ultracentrifuge, SW-41 bucket-rotor, 20°C, 39,000 rpm, 2.5 h). a) DNA from loach sperm; b, c) DNA from loach sperm, but treated with proteinase K (Serva, Germany 40 g/ml, 24 h, 37°C) and phenol before thiol incubation; d, e) DNA from loach erythrocytes, but treated with proteinase K and phenol before thiol-incubation; f, g) DNA from hens' erythrocytes, but treated with RNase A (Merck, Germany, 40 g/ml, 24 h, 37°C) and proteinase K before thiol incubation; h, i) DNA from hens' erythrocytes, but treated with proteinase K and phenol before thiol incubation. 1) control (without ME), 2-4) ME, pH 4.4, 5.3, and 5.9 respectively; 5, 6) pH 8.0, 0.025 M EDTA, ME, and DTT respectively. Incubation with thiols (0.1%) at 37°C for 5 days (a, b, d, f, h) and 10 days (c, e, g, i).

Fig. 2. Melting of supramolecular DNA complex of loach sperm under normal conditions and after incubation with 0.1% ME for 5 days (A) and 10 days (B). 1) control (without ME); 2-4) ME at pH 5.9, 5.3, and 4.4 respectively. ordinate, hyperchromic effect (in per cent). DNA treated with proteinase K and phenol before incubation with ME.

order to determine the limits of thiol-induced fragmentation of the complex, we used long-term incubation with thiols (5 and 10 days in the presence of 5 μl of diethylpyrocarbonate as nuclease inhibitor), followed by sedimentation in a neutral sucrose gradient. It will be clear from Fig. 1 that, depending on the experimental conditions, a DNA complex split up into subunits of different sizes. Limiting degradation of the DNA complex of three eukaryote cells was found to take place with ME at pH 4.4 for 5 days, with virtually no increase by 10 days. The size of the subunits was $5 \cdot 10^5$ D, commensurate with the gene (at pH 5.9 it was $7.8 \cdot 10^6$ D). However, ME, pH 8 + EDTA, even acting for 10 days, did not change the sedimentation constant of the DNA complex, although it completely destroyed its viscoelasticity, which is responsible for the rosettelike structures of DNA (not shown). Meanwhile, DTT, pH 8 + EDTA caused more profound degradation of the complex than ME under the same conditions, namely to subunits with mol. wt. of $(1\text{-}2) \cdot 10^7$ D, commensurate with a transcripton. Conversely, sodium borhydride at pH 8, which reduces.only free S–S groups [9], did not in general induce fragmentation of the complex (not shown), in agreement with absence of the effect of sodium bisulfite on the DNA complex in our earlier study [4] and is evidence in support of the presence of metal in the bisulfide protein cross-linkages (S–Me–S), as was suggested in [9].

It is important to note that additional treatment of DNA complexes from loach sperm and erythrocytes with proteinase K and phenol before incubation with thiols, in order to remove contaminating nucleases, did not affect the sedimentation constant of the thiol-induced subunits (Fig. 1). Meanwhile ME at acid pH values (4.5-5.9) had a weaker effect on the DNA complex from hens' erythrocytes, pretreated with RNase A and proteinase K than on the DNA complex from loach sperm and erythrocytes. However, if the DNA complex of hen's erythrocytes was pretreated with proteinase K and phenol, more profound ME-induced fragmentation was observed at pH 5.3 and 5.9, although it was less marked than after similar treatment of the loach DNA complex. This can be explained by differences in accessibility of the S-S groups in these DNA preparations for thiols.

In our view thiol-induced fragmentation of the supramolecular DNA complex is the result of reduction of protein disulfide cross-linkages, participating in the quasisubunitary organization of DNA. The following facts are opposed to the possible participation of contaminating nucleases, reanimated by thiols, in degradation of DNA. Bivalent metals (10 mM $MgCl_2$, 5 mM $CaCl_2$) at pH 5 inhibited thiol-induced degradation of the complex. ME in neutral medium, but without EDTA, did not induce degradation even in the course of 10 days (not shown). Pretreatment of the DNA complex with proteinase K and phenol did not affect the size of the thiol-induced DNA subunit (Fig. 1).

To elucidate the nature of thiol-induced fragmentation of the supramolecular DNA complex, melting curves of the ME-induced subunits obtained after long-term incubation with thiols at acid pH values were studied. It will be clear from Fig. 2 that ME-induced fragmentation of the loach sperm DNA complex pH 4.4 was accompanied by a shift of the melting temperature into the region of lower values and by reduction of the hyperchromic effect of the subunits, evidence of the existence of 'rsticky" ends. It can accordingly be concluded that ME-induced fragmentation of the DNA complex takes place as a result of a "slanting" double-stranded break. Calculation shows~that on average 180 ± 11 bp are involved in such a break. This value is characteristic of all DNA preparations and has been reliably tested for subunits measuring $(0.5\text{-}2.0) \cdot 10^6$ D. Thus not only S–S protein cross-linkages, but also complementary "sticky" ends of subunits can take part in tandem combination of eukaryote DNA subunits. It has been suggested [12] that tandem combination of two subunits is affected with the aid of four polypeptides, joined together in pairs through S–S cross-linkages, and that these pairs of polypeptides are arranged in antiparallel DNA strands, 180 bp apart. It is probable that "sticky" end regions may be sites of interaction with DNA topoisomerase II. Evidence in support of such a possibility is given by data showing that it is DNA topoisomerase II (and not topoisomerase I) which forms disulfide-linked oligomers [11] and can perform a flanking role between discrete DNA fragments [2].

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