ALTERATION OF THE DNA SECONDARY STRUCTURE IN THE DNA–POLYLYSINE COMPLEX EVIDENCED BY SODIUM BISULFITE MODIFICATION

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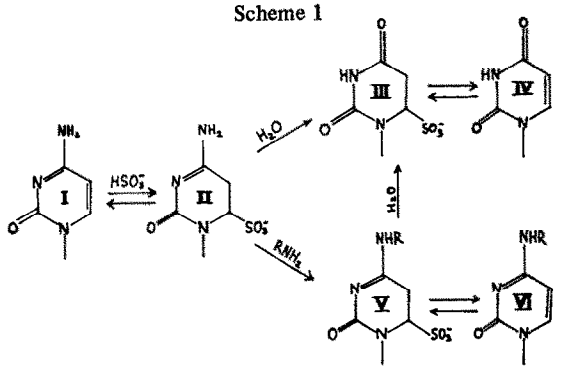
1. Introduction

The results of numerous physico–chemical and biochemical studies of the bacteriophage structures show that the DNA tightly coiled inside the bacteriophage heads has regions with disordered base stacking though the ordered B conformation of the sugar-phosphate DNA backbone is conserved [1]. The results of chemical modification of the intraphage DNA with nucleophilic agents showed that the regions with the disordered base stacking contained 18% of total cytosine [2–4]. The nucleophilic agent sodium bisulfite attacks the $C_5 = C_6$ bond of cytosine in the direction perpendicular to the plane of the heterocyclic ring (see scheme 1) only in the bases free of the stacking interaction; therefore, the bisulphite reaction is sensitive to the secondary structure of the polynucleotides [5]. We have shown [3,4] that modification of the intact S_d phage with bisulfite results in formation of DNA–protein crosslinks in the form of ϵN-(2-oxopyrimidyl-4)-lysine (scheme 1, product VI).

Scheme 1



RNH_2 = amino acid or peptide with free amino group

These results suggest that the peculiar structure of the intraphage DNA with the partially disordered base stacking is determined by its compaction in the phage head and specific interaction with the phage proteins involving the side groups of lysine. In this connection, it is interesting to look for similar structural changes of the DNA and the DNA–protein crosslinks in other compact forms of deoxyribonucleoproteins following bisulfite modification. For this purpose we have studied bisulfite modification of DNA in the complex with polylysine which has a compact toroidal structure similar to the structure of the intraphage DNA [6].

Our results show that a considerable part of cytosine residues in the DNA–polylysine complex is accessible for bisulfite modification which results in formation of cytidyl–lysine crosslinks.

2. Materials and methods

The DNA preparations were obtained by phenol deproteination [7] of the S_d bacteriophage grown and purified as in [8]. The equal amount of the poly(L-lysine) HBr solution (Serva, mol. wt 6000–9000) with the concentration of 1.5 mM (315 μg/ml) was rapidly added to the 1.5 mM (450 μg/ml) DNA solution to obtain the 1:1 lysine–nucleotide ratio. The final NaCl concentration of the solution was 0.5 M. After mixing and vigorous stirring the complex appeared as a filamentous aggregate. The complex was stored at room temperature for 2 h.

For modification the equal volume of 0.5 M $Na_2S_2O_5$ solution (pH 6.5) was added to the solution of the DNA–polylysine complex. Commercial

bisulfite was used after ethanol recrystallization from deionized water. The mixture was incubated for 24 h at 25°C in dark. The reaction was stopped and bisulfite was removed as in [3,4]. The modified complex was digested by 57% $HClO_4$ and reaction products were identified by thin-layer chromatography as described for bisulfite modification of S_d phage [3,4].

3. Results

We have shown [3,4] that after $HClO_4$ digestion the cytosyl–amino acid products of bisulfite modification of the intraphage DNA (scheme 1) can be readily separated from the free bases by gel-filtration on a Biogel P-4 column (100–200 mesh). The percentage of the modified cytosine residues can be estimated by subsequent thin-layer chromatography of the bases eluted from the column.

Figure 1 shows the elution profile for the digestion products of the modified DNA–polylysine complex and fig. 2 presents the ultraviolet spectrum of the material eluted in the free volume (peak 1). For comparison fig. 2 shows the ultraviolet spectra of a similar material obtained after bisulfite treatment of intact S_d phage and the spectra of the model ϵN-(2-oxopyrimidyl-4)lysine. Recorded spectra show a characteristic peak shift and the absorption increase on acidification testifying to the presence of cytosine residues in the material from the modified DNA–polylysine complex, similarly to the material from the modified S_d phage eluted from the Biogel P-4 in the free volume.

The electrophoretic and chromatographic mobilities of the material from the first peak of the modified complex given in table 1 suggest that the material

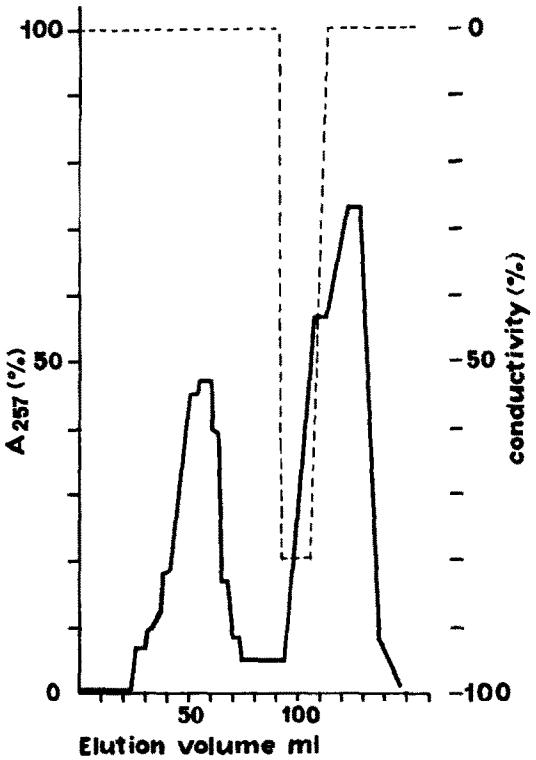


Fig. 1. Chromatographic profile for the $HClO_4$ digestion products of the bisulfite-modified DNA–polylysine complex on biogel P-4 (100–200 mesh). (——) A_{276} nm; (- - -) electric conductivity.

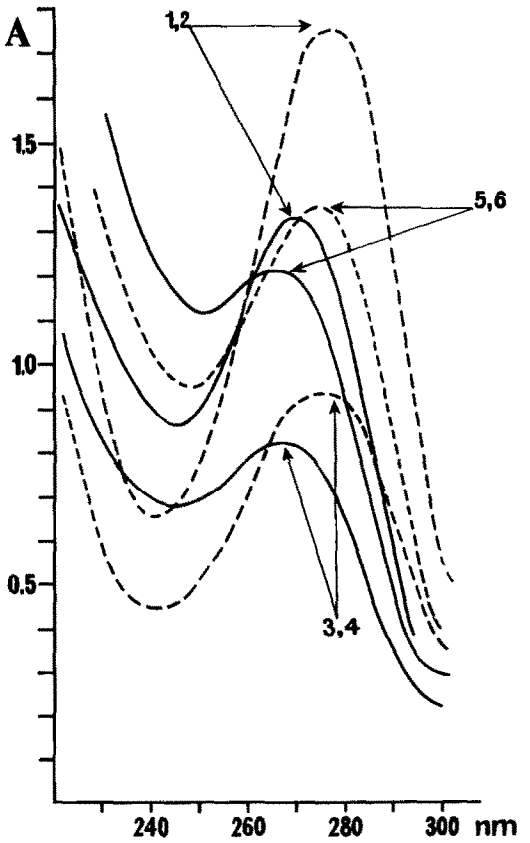


Fig. 2. Absorption spectra of the material from the modified DNA–polylysine complex and the modified phage S_d. (——) in H_2O; (– – –) in 0.1 N HCl; (1, 2) DNA–polylysine complex; (3, 4) phage S_d; (5, 6) model cytosyl–lysine.

Table 1
Chromatographic and electrophoretic mobilities of the modification products of the DNA in the DNA–polylysine complex and in the phage S_d

Material	Chromatographic mobility in systems			Electrophoretic mobility
	a	b	c	d
Modification products of DNA–polylysine complex	0.05	0.30	0.85	0.61
Modification products of DNA in phage S_d	0.05 0.45	0.30	0.85	0.61 0.18 0.09[a]
Cytosine	0.26	0.56	0.70	1.00
Uracil	0.38	0.67	0.70	0.00
Cytosyl–lysine	0.055	0.30	0.85	0.61
Cytosyl–alanine	0.13	0.30	0.85	0.26

[a] Migration towards the anode; the rest - towards the cathode

Note: System (a) Butanol:water (86:14) [9]; (b) Methanol:HCl:water (70:20:10) [10]; (c) 5% Na_2HPO_4, saturated with isoamyl alcohol (water phase); (d) in 7% CH_3COOH relative to cytosine

(peak I) whose ultraviolet spectra are given in fig. 2 (curves 5, 6) is cytosyl–lysine since all its characteristics are identical with those of the authentic cytosyl–lysine and cytosyl–lysine product isolated from the modified S_d phage.

To estimate the degree of modification of the DNA in the DNA–polylysine complex we determined the base composition of the bisulfite-treated free S_d DNA and the modified DNA–polylysine complex. The base composition was determined by thin-layer chromatography after separating the appropriate $HClO_4$ digestion products on the biogel P-4 [3,4] (see table 2). In contrast to the native DNA in solution, the DNA in the complex was found to contain 40% less cytosine. No noticeable amount of uracil was found.

Thus, our results show that incubation of the DNA–polylysine complex with bisulfite produces considerable (40%) modification of cytosine. The reaction, according to scheme 1, results in transamination of the amino group of cytosine, i.e., in the formation of ϵN-(2-oxypyrimidyl-4-lysine) (see section 1).

Hence, the DNA in the complex has the same properties with respect to the nucleophilic agents as the DNA in the phage head, i.e., a considerable part of cytosine is not involved in the base stacking and the

Table 2
Nucleotide composition (in mol %) of the modified DNAs in solution and in the DNA–polylysine complex determined after separation of the respective $HClO_4$ digestion products on the biogel

Bases	DNA		DNA–polylysine complex	
	Exp.I	Exp.II	Exp.I	Exp.II
Adenine	28.1%	28.8%	30.1%	31.8%
Thymine	28.3%	28.6%	33.4%	36.2%
Guanine	21.4%	20.6%	22.9%	22.2%
Cytosine	22.2%	22.0%	13.6%	9.8%
Uracil	0	0	0	0

$C_5 = C_6$ bond of cytosine is accessible for nucleophilic attack.

4. Discussion

The X-ray diffraction [6] and Raman spectroscopy [12] results demonstrate that the DNA in the compact toroidal DNA–polylysine complex has the ordered B conformation of the sugar–phosphate backbone. At the same time, ^1H / ^3H exchange studies for the DNA–polylysine complex show that ~1/4 of the hydrogen bonds between the DNA bases in this complex are broken and according to Raman data [12] a considerable part of the base stacking interaction in the DNA–polylysine complex is disrupted (mainly in the A–T pairs). Our results on bisulfite modification show that a considerable part of cytosine bases in the DNA–polylysine complex is also not involved in the base stacking. Thus, the structural changes of the DNA in the complex with polylysine in the form of disruption of base stacking involve not only A–T but also G–C basepairs, that is, a considerable part of the entire DNA molecule.

Despite numerous studies, it is not yet definitely known in which of the DNA grooves the polylysine chains are located in the case of stoichiometric (lysine:nucleotide = 1:1) binding with the DNA [14,15]. Our results on crosslinking by transamination of the ϵ-amino group of lysine and C_4 $-NH_2$ group of cytosine after bisulfite modification of the DNA–polylysine complex provide the direct evidence that in the complex a considerable part of the ϵ-amino group of lysine is located in the major groove of the DNA since the C_4 $-NH_2$ cytosine group is located in the major groove of the DNA double helix. Thus, if the polylysine chains in the complex are located in one of the DNA grooves it should be the major groove.

The above data on the structure of the DNA–polylysine complex reveal a considerable similarity between the structure of the intraphage DNA and DNA in the complex with polylysine. The X-ray diffraction results demonstrate the B conformation of the DNA in the phage heads [16]. At the same time, our infrared spectroscopic [17,18] and Raman results for the intravirus DNA (to be published) show that the sugar–phosphate backbone of the intraphage DNA has the ordered B conformation but the stacking interactions of the bases are considerably disordered. Finally, 18% of the cytosine in the intraphage DNA is unstacked and can be chemically modified by nucleophilic agents giving rise to cytosyl–lysine crosslinks.

All the above data on the DNA structure in phages and in the DNA–polylysine complex lead to a suggestion that the special DNA structure with partially disordered base stacking but retaining the ordered B conformation of the sugar–phosphate backbone is typical of the compact form of the DNA in the deoxyribonucleoprotein complexes. Further studies are needed to find out whether these structural alterations result from compaction of the DNA or are caused by specific DNA–protein interactions involving the side groups of lysine.

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References

[1] Tikchonenko, T. I. (1975) in: Comprehensive Virology (Fraenkel-Conrat, H. and Wagner, R. R. eds) vol. 3, pp. 1–117, Plenum, London, New York.
[2] Tikchonenko, T. I., Budovsky, E. I., Sklyadneva, V. B. and Chromov, I. S. (1971) J. Mol. Biol. 55, 535–547.
[3] Sklyadneva, V. B., Chekanovskaya, L. A., Nikolaeva, I. A. and Tikchonenko, T. I. (1978) Biochimiya 43, 1563–1577.
[4] Sklyadneva, V. B., Chekanovskaya, I. A., Nikolaeva, I. A. and Tikchonenko, T. I. (1979) Biochim. Biophys. Acta in press.
[5] Hayatsu, H. (1976) Prog. Nucleic Acid Res. Mol. Biol. 16, 75–124.
[6] Haynes, M., Garrett, P. A. and Gratzer, W. B. (1970) Biochemistry 9, 4410–4416.
[7] Tikchonenko, T. I., Koudelka, J. and Borispolets, S. I. (1963) Mikrobiologiya 32, 723–726.
[8] Tikchonenko, T. I. (1963) Biochimiya 27, 1015–1022.
[9] Hotchkiss, R. D. (1948) J. Biol. Chem. 175, 315–317.
[10] Kirby, K. S. (1955) Biochim. Biophys. Acta 18, 575–579.

[11] Laster, P. and Blair, A. (1963) J. Biol. Chem. 238, 33–48.
[12] Prescott, B., Chou, C. H. and Thomas, G. J., jr (1976) J. Phys. Chem. 80, 1164–1177.
[13] Lees, C. W. and Von Hippel, P. H. (1968) Biochemistry 7, 2480–2488.
[14] Wilkins, M. H. F. (1956) Cold Spring Harbor Symp. Quant. Biol. 21, 75–90.
[15] Tsuboi, M., Matsui, K. and T'so, P. O. P. (1966) J. Mol. Biol. 15, 256–267.
[16] Feigin, L. A., Dembo, A. T. and Boyarintseva, A. K. (1974) J. Appl. Crystalog. 7, 164–167.
[17] Shie, M., Chirgadze, Yu. N. and Tikchonenko, T. I. (1970) Vopr. Virusol. 5, 619–622.
[18] Shie, M., Kharitonenkov, I. G., Tikchonenko, T. I. and Chirgadze, Yu. N. (1972) Nature 235, 386–388.