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A compact form of methylated DNA in solutions containing poly (ethylene glycol)

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ABSTRACT

Some peculiarities of compactization of double-stranded DNA molecules containing methylated nitrogen bases have been studied in water-salt solutions of PEG. It is shown that the methylation of N^7 - atoms of guanyl residues in original DNA molecules does not prevent the formation of DNA compact particles, but results in a decrease of the amplitude of the negative band in the CD spectrum of compact particles. The influence of N^7 - guanine methylation on the shape of the CD spectrum being the greater, the lower is the concentration of PEG. The dependence of the negative band amplitude in the CD spectrum on the content of methylated guanyl residues is practically the same for low-molecular weight DNA's from different sources. The observed decrease in the negative band amplitude is interpreted as a result of alteration of guanyl residue orientation relative to the helix axis which leads to diminished optical activity of the "microcrystalline" domains of compact particles. The evidence obtained suggests that changes in the secondary structure of DNA lead to considerable differences between CD spectra of compact particles of methylated DNA and Ψ - form of DNA. (The changes in the CD spectrum of the DNA compact particles occur also as a result of methylation of C^5 - atoms of cytosine residues). It is suggested that the negative band in the CD spectrum can be used as a criterion for detection of negligible alterations in the DNA secondary structure.

INTRODUCTION

It has been shown [1-4] that the addition of poly(ethylene glycol) to neutral water-salt solutions of double-stranded polynucleotides (DNA,RNA) results in the formation of compact particles, the particles formed from the native double-stranded DNA or RNA being characterized by a regular specific packing of the polynucleotide chain (Ψ - form of DNA or RNA). Such specific packing may be one of the reasons of the appearance of an intense band in the CD spectrum. It has been suggested [5-7] that the packing of the polynucleotide chain and, consequently, the intense band in the CD spectrum are affected by alteration in the parameters of the secondary structure of the polynucleotides. This suggestion is borne out by the results of studies on the compactization of DNA in water-salt solutions of different acidity. It

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has been shown that while the protonation of nitrogen bases does not prevent the formation of compact particles, it leads to a decrease of the specific CD band as compared to the case of Ψ- form of DNA [6].

The purpose of this study was to elucidate the correlation between the specificity of DNA packing in a compact particle, the degree of defectiveness of its secondary structure, and the peculiarities of the CD spectrum. For this purpose compact particles were formed from DNA molecules with different content of methylated bases and their CD spectra were compared.

MATERIALS AND METHODS

DNA from Streptomyces chrysomallus and calf thymus have been methylated chemically (by means of dimethylsulfate) in 2M NaCL in the presence of tri-n-butylamine according to the procedure described in details [8]. The procedure used assures incorporation of the methyl group predominantly into N^7-position of guanine. To evaluate the degree of methylated bases, DNA was hydrolyzed with I N HCL during 20 min at IOO^oC followed by fractionation of hydrolytic products on Dowex 50x8 or IR I20 columns. The compositions of methylated DNA's are given in table I. No methylated adenine could be detected in the samples by this method.

The melting curves of methylated DNA were sharp, but the melting temperature decreases with increasing amount of methylated guanine. (For example, for DNA Streptomyces chrysomallus $T_m \sim 84^oC$ (0% MeG; 0.02 M $NaClO_4$) and $T_m \sim 75^oC$ (42% MeG; 0.02 M $NaClO_4$). In accordance with previous data [8], lowering of T_m indicates the alterations in the base-base interactions and in the repulsion forces between phosphate residues due to the formation of positively charged MeG-sites.

In order to prevent the hydrolysis of methylated DNA samples all solutions were stored at + 4^oC.

DNA samples of molecular weights smaller than that of original samples were obtained by ultrasonic depolymerization at + 0.4^oC (using a УЗДН disintegrator, 35 kHz, sonication time from 3 to 30 sec). After the depolymerization DNA solutions were dialyzed against 0.3 M NaCL. The molecular weights of DNA samples were determined from their sedimentation coefficients using the formula of Crothers - Zimm [9] (table I).

In titration of DNA with distamycin A ($\lambda_{303\ nm}$ = 30,000; "Calbiochem", USA) I.0 μl of distamycin ($2.5x10^{-3}$; H_2O) was added to I.8 ml of DNA (I5 μg/ml; 0.3M NaCL)[7]. The resulting titration curves (which reflect the binding of distamycin to AT-regions of DNA) are presented in Fig.I as a

Table I. Characteristics of the DNA samples

Str. chrysomallus DNA (72% GC)		Calf thymus DNA (42% GC)	
content of MeG (%)	mol.weight	content of MeG (%)	mol.weight
0	6.30×10^6 0.51×10^6 0.26×10^6 0.20×10^6	0	12.60×10^6 0.51×10^6 0.26×10^6 0.20×10^6
24	6.30×10^6 0.51×10^6 0.26×10^6 0.20×10^6	20	12.60×10^6 0.51×10^6 0.26×10^6 0.20×10^6
42	6.30×10^6 0.80×10^6 0.33×10^6 0.20×10^6	35	12.60×10^6 1.00×10^6 0.33×10^6 0.20×10^6

dependence of amplitude of the positive band in the CD spectrum (λ = 330 nm) on "r" ("r" - the number of moles of distamycin per mole of DNA base pairs).

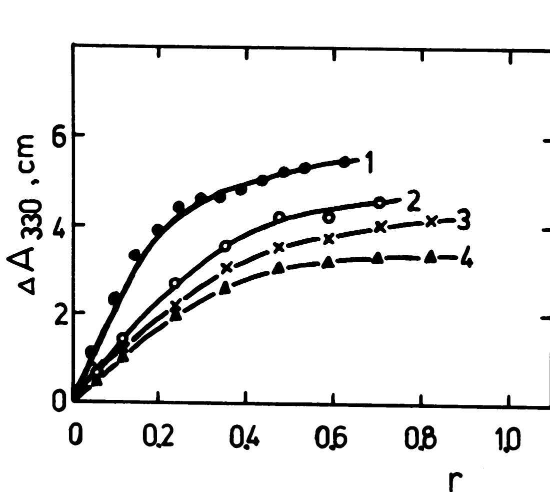

Fig.I. Dependence of amplitude of the positive band (ΔA_{330}) in the CD spectrum upon distamycin A added (calf thymus DNA, content of MeG in DNA: curve I-0; 2-4; 3-20; 4-35%; mol. wt of DNA = 0.32×10^6);

The C^5-cytosine methylation has been performed *in vivo* [10]. DNA preparations containing methylated C^5-cytosine were isolated from rat liver 8-10 and 24 hours after the intravenous injection of 4-methyl-2,6-di-t.-butylphenol ("ionol"). The content of methylated cytosine was determined as described[10].

To form DNA compact particles in PEG-containing solutions (mol. wt 20,000; "Merck", West Germany) the procedure described previously in details was used [3,5]. In order to avoid intermolecular aggregation the final concentration of DNA in the solutions prepared was 5 μg/ml; the concentrations of PEG were 80, 100 and 150 mg/ml.

The formation of compact particles in all cases was demonstrated by the

apparent optical density in the DNA absorption spectrum [II] as well as by electron microscopy [3].

The absorption spectra of DNA water-salt solutions in the absence and in the presence of PEG were recorded with a "Beckman" spectrophotometer (USA). As an example, Fig.2 shows the absorption spectra of calf thymus DNA (35% MeG) in water-salt solutions in absence and in the presence of PEG.

The appearance of absorption at λ >320 nm points to the formation of large DNA particles in PEG-containing solutions ($C_{PEG} \geq 80$ mg/ml) in spite of modification of DNA molecules. Similar changes were observed in the absorption spectra of Str. chrysomallus DNA.

The CD spectra of PEG-containing DNA solutions were recorded with a "Roussel-Jouan CD-I85" dichrograph (France) using 3 cm cells.

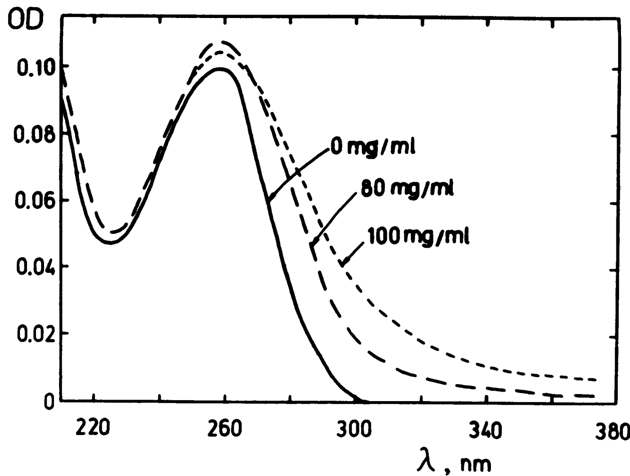

Fig.2. Absorption spectra of calf thymus DNA (35% of MeG) in water-salt solutions (0.3 M NaCL) in the absence of PEG and in the presence of 80 and I00 mg/ml of PEG. (Mol. weight of DNA - I2.60xI0^{6}).

RESULTS AND DISCUSSION

Fig.3 compares, as an example, CD spectra of solutions of methylated DNA's from Str. chrysomallus and from calf thymus in presence of PEG. Each series of spectra corresponds to DNA samples having the same content of MeG and differing only in molecular weight. This figure shows that there is a complex dependence between the negative band amplitude ($\Delta\varepsilon_{270}$) and the molecular weight: as the molecular weight decreases, the amplitude at first increases to reach a maximum and then decreases. The dependence obtained coinside with previous results [I2], where the dependence of $\Delta\varepsilon_{270}$ on mol. weights of double-stranded DNA has been measured thoroughly. A dependence of $\Delta\varepsilon_{270}$ upon molecular weight of DNA shown in Fig.4 indicates that the maxi-

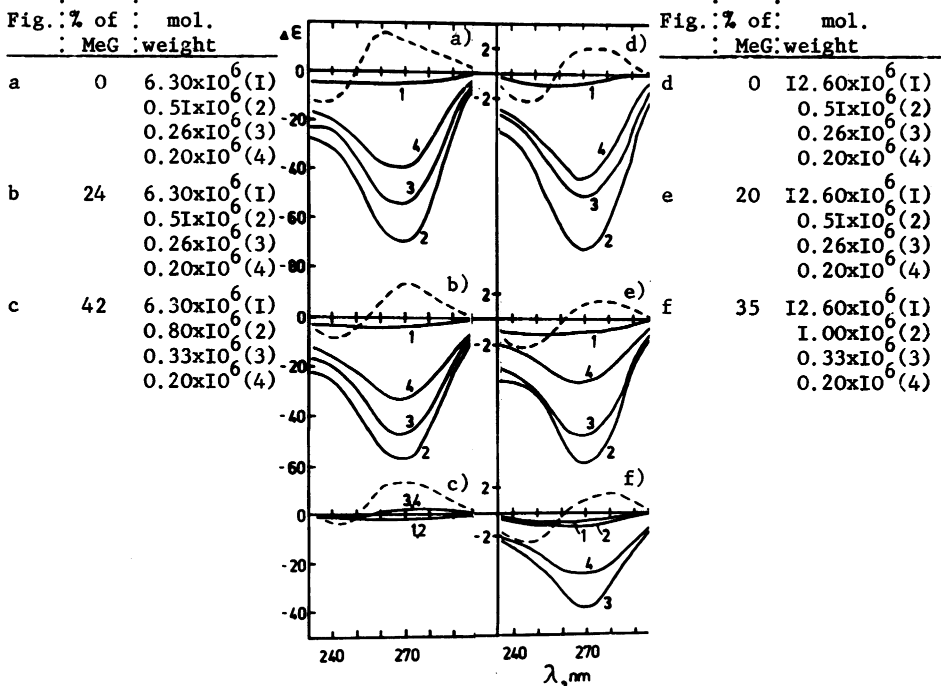

Fig.3. CD spectra of Str. chrysomallus DNA (a-c) and of calf thymus DNA (d-f) with different content of MeG in water-salt solution (0.3 M NaCL) containing I00 mg/ml of PEG. Content of MeG and mol. weights of samples - see left and right panels. The dotted line indicates the CD spectra in the absence of PEG (the ordinate is in the centre of the figure).

mum in $\Delta\varepsilon_{270}$ corresponds to molecular weight of $\sim 5\times10^5$. This "critical" value of molecular weight is independent of the nature of DNA samples used and of the percentage of methylation. The existence of critical molecular weight indicates that compact DNA particles with high optical activity (Ψ- form of DNA) can be formed from molecules whose lenght is close to 500 base pairs. It should be noted that the existence of a "critical" length of DNA required for the formation of the optically active compact particles is supported by data on enzymatic cleavage of the DNA molecule forming a compact particle [I3] as well as by the results reported [I2,I4].

Comparison of CD spectra of DNA samples having the same molecular weight and differing in the content of MeG (see Fig.3) points to a considerable effect of methylation on the negative band amplitude ($\Delta\varepsilon_{270}$), this effect being the greater, the smaller the value of C_{PEG}. The dependence of $\Delta\varepsilon_{270}$ on content of MeG for Str. chrysomallus and calf thymus DNA's (mol.wt

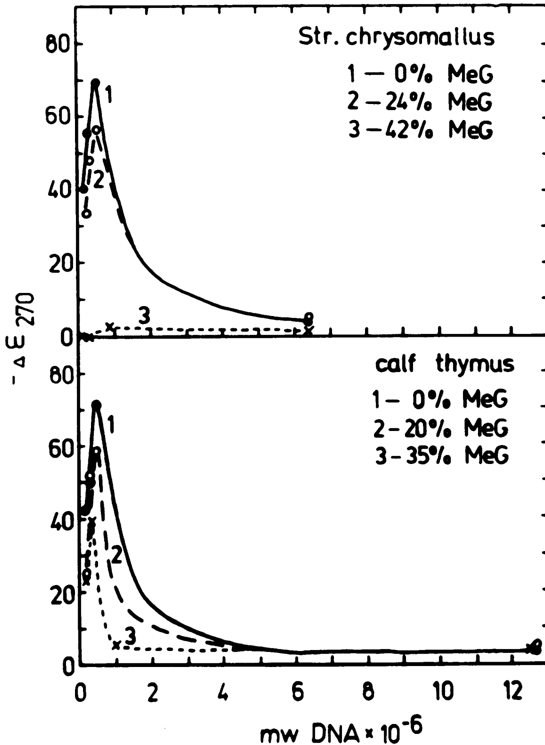

Fig.4. Dependence of the amplitude of the negative band ($\Delta\varepsilon_{270}$) in the CD spectrum on the molecular weight of DNA (100 mg/ml of PEG; 0.3 M NaCL).

$\sim 5 \times 10^5$) shown in Fig.5 indicate that the negative band intensity diminishes as the percentage of MeG in DNA is increased. However, the dependence of $\Delta\varepsilon_{270}$ on content of MeG is practically the same for DNA samples from different sources.

There may be two possible explanations for the observed decrease of the negative band amplitude in the CD spectrum of compact particles with increase of MeG. First, methylation of DNA may prevent the formation of compact particles as result of considerable denaturation of DNA. Second, the methylation of N^7-guanine may lead to a change in the stacking interaction and in the interaction with other bases within "microcrystalline" domains is compact particles (formed at a given concentration of PEG) and, consequently, to a change in the contribution of GC base pairs to the optical activity on the compact particle. It means that the compact particles formed from methylated DNA molecules are characterized by CD spectra with decreased negative band amplitude despite the ordered packing of the DNA chain in compact particles.

Our measurements of absorption spectra (see Fig.2) indicate that

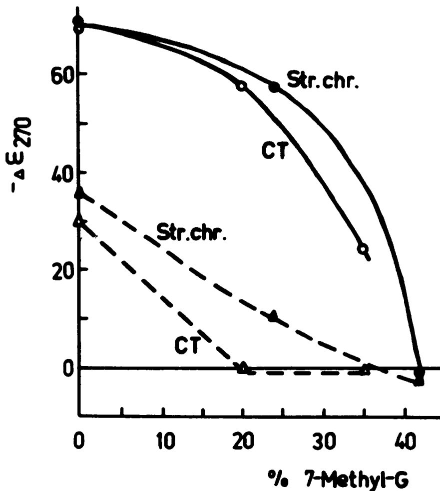

Fig.5. Dependence of the amplitude of the negative band ($\Delta\varepsilon_{270}$) in the CD spectrum on the content of MeG in Str. chrysomallus and calf thymus (CT) DNA's (mol.wt of DNA ~ $5x10^5$; C_{PEG}= 80 mg/ml (dotted lines) and 100 mg/ml (solid lines); 0.3 M NaCL).

methylated DNA molecules may form particles in PEG-containing solutions ($C_{PEG} \geqslant 80$ mg/ml). Electron microscopy of methylated DNA molecules in PEG-containing solutions revealed toroid-shaped particles with an external diameter of about 1600 Å (Fig.6). The shape of the compact particles formed from methylated DNA molecules is very similar to the shape of the compact particles of Ψ-form of DNA. These results therefore rule out the first alternative and favour the second one. Consequently, the methylation of DNA molecules does not prevent the formation of compact particles in PEG-containing water-salt solutions. The supposition that the decreased negative band amplitude in the CD spectrum is due to alteration (but not denaturation) of GC base pairs in the double helix as result of methylation, is also confirmed by the fact that at C_{PEG} = 80 mg/ml, i.e., when the number of optically active "microcrystalline" domains formed in compact particles is still small [15], the methylation of small number of guanyl residues is sufficient to "inhibit" the optical activity of compact particles (Fig.7).

It should be noted also that it is only at C_{PEG} slightly exceeding the "critical" concentration of PEG, which is necessary for formation of compact particles, the $\Delta\varepsilon_{270}$ value is dependent on the nucleotide composition: the higher the content of GC base pairs, the higher the value of $\Delta\varepsilon_{270}$. However, this dependence may be not connected with a larger contribution of GC base pairs (in comparison with AT base pairs) to the negative band intensity, but rather indicates that GC-rich DNA's form "microcrystalline" domains in compact particles at lower PEG concentrations [14,16].

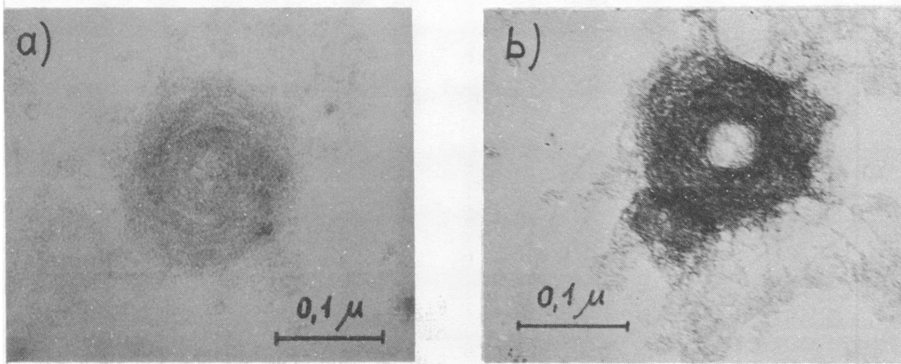

Fig.6. Electron micrographs (a) of DNA Str. chrysomallus DNA (42% of MeG, 150 mg/ml of PEG) and (b) of calf thymus DNA (35% of MeG, 100 mg/ml of PEG),

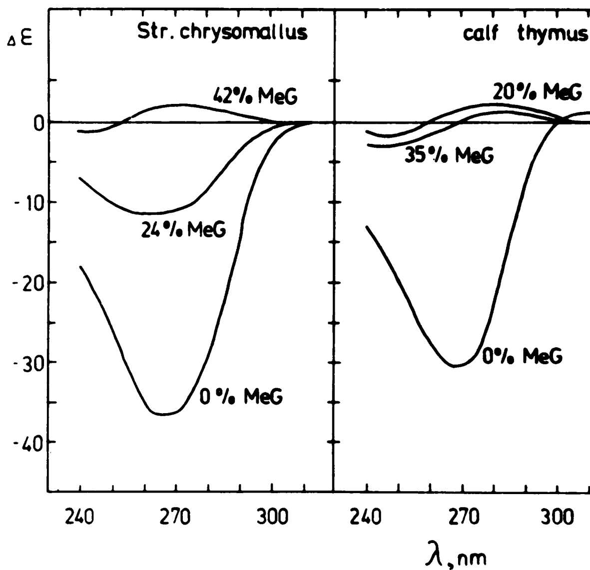

Fig.7. CD spectra of Str. chrysomallus and calf thymus methylated DNA's (mol.wt ~ $5x10^5$) in water-salt solution (0.3 M NaCL) containing 80 mg/ml of PEG.

The dependence of $\Delta\varepsilon_{270}$ value on DNA nucleotide composition disappears when the PEG concentration is increased up to 150 mg/ml, i.e. under conditions when a greater proportion of DNA base pairs is involved in the formation of "microcrystalline" domains. The increase in the total number of these domains leads to a situation where the optical activity of a

compact particle is no longer "inhibited" even at the highest degree of guanine methylation used in this study, so that compact particles having an intense negative band in their CD spectra can again be formed (Fig.8).

It should be pointed out, however, that although a negative band does appear in the CD spectrum of methylated DNA at 150 mg/ml, it is less intense than that characteristic of nonmethylated DNA of the same molecular weight under similar conditions. Decreased intensity of the CD band may be explained by deformation of DNA secondary structure at sites of guanine methylation [8,17,18], which leads to a change of interaction between base pairs within the "microcrystalline" domains in compact particles.

To verify whether the methylation of GC base pairs influence the state of neighbouring AT base pairs in the molecule of DNA, the compactization of methylated DNA was studied in the presence of distamycin A. It has been shown [7] that the binding of distamycin A to AT-rich regions in DNA molecules is accompanied by such changes in DNA secondary structure, which leads to the formation of compact particles with an intense positive band in the CD spectrum. The shape of the positive band in the CD spectrum is similar to the shape of the spectra of compact particles formed from double-stranded RNA molecules (which belong to A-family of polynucleotides) [4]. The positive

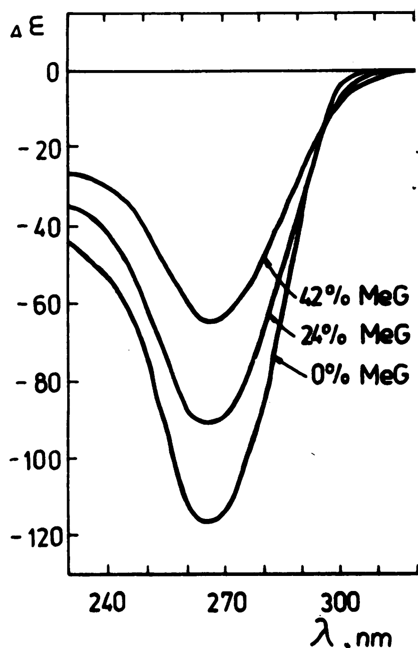

Fig.8. CD spectra of methylated Str. chrysomallus DNA (mol.wt ~ $5x10^5$) in water-salt solutions (0.3 M NaCL) containing 150 mg/ml of PEG.

band in the CD spectrum of distamycin-bonded DNA molecules being the greater, the higher is AT-content of DNA molecules. The amplitude of this positive band in the CD spectrum of compact particles ($\lambda \sim$ 265-270 nm) formed from distamycin-bonded DNA molecules is rather similar in magnitude to that of the negative band in the CD spectrum of compact particles formed from calf thymus DNA in a PEG-containing solution in the absence of distamycin A (Fig. 9).

The observation that the positive band in the CD spectrum of compact particles formed in the presence of distamycin A disappears at a lower level of MeG within DNA than the negative band is indicative of altered orientation not only GC base pairs but also neighbouring AT base pairs as result of N^7-guanine methylation (compare curves 3 and 3', Fig. 9).

Data shown in Fig. 9 indicate that the CD spectra of compact particles formed from DNA molecules with high optical activity (mol.wt $\sim 5x10^5$) may

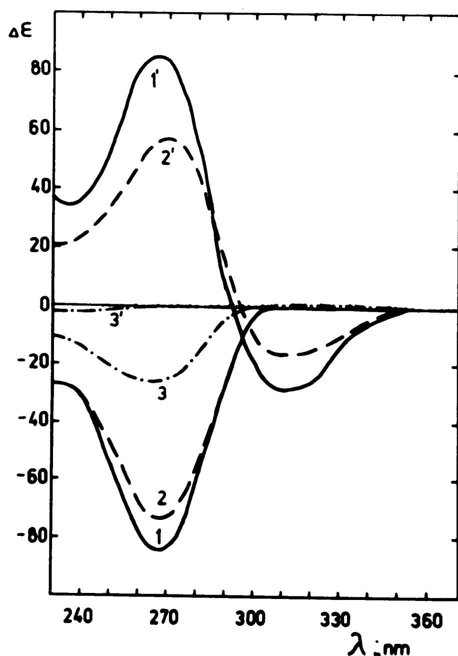

Fig. 9. CD spectra of calf thymus DNA (mol.wt $\sim 3x10^5$) in water-salt solution (0.3 M NaCL) containing 100 mg/ml of PEG in the absence (1,2,3) and in the presence (1',2',3') of distamycin A (content of MeG in DNA: curve 1 and 1' - 0; 2 and 2' - 4; 3 and 3' - 20%).

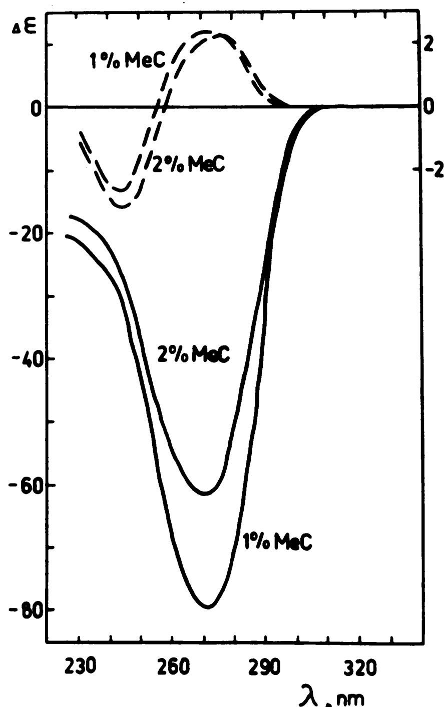

Fig. 10. CD spectra of rat liver DNA containing 1 and 2 % of methylcytosine in a water-salt solution (0.3 M NaCL) containing 150 mg/ml of PEG (dashes show CD spectra of DNA in the absence of PEG - the right ordinate).

be used as a criterion for detection of small alterations in the secondary structure of DNA. The use of DNA samples with high molecular weight for these purposes is not effective, because the intensity of the bands in the CD spectrum of such particles is very small.

The negative band in the CD spectrum decreases not only in the case of compact particles formed from N^7-guanine methylated DNA, but also in the case of such particles formed from C^5-cytosine methylated DNA molecules (Fig.IO). The more pronounced effect of methylation of cytosine than of guanine (cf. Fig.8 and Fig.IO) is rather difficult to interpret at this stage.

It should also be noted that the change in band intensity in the CD spectrum of compact particles formed from protonated DNA [5,I5] appears to differ only quantitatively from the change in band intensity of the CD spectrum of compact particles of methylated DNA, because the protonation of bases involves both GC and AT base pairs, i.e. it leads to a more "rapid" disappearance of the negative band in the CD spectrum of compact particles formed from protonated DNA [I9].

The present results thus show that the introduction of "defects" into the secondary structure of DNA by the way of methylation of nitrogen bases strongly changes the optical properties of compact particles formed from methylated DNA molecules.

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[x)]Abbreviations used are: PEG - poly(ethylene glycol); C_{PEG} - concentration of PEG; CD - circular dichroism; MeG - N^7-methylguanine.

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