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Spermine-DNA complexes build up metastable structures. Small-angle X-ray scattering and circular dichroism studies

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ABSTRACT

Spermine-DNA complexes have been examined by small-angle and wide-angle X-ray scattering as well as by circular dichroism studies.
Condensed complexes are building up below a critical ionic strength. We have found that at one and the same ionic strength condensed complexes having two different supramolecular structures (Type I and Type II) can coexist. The structure of the condensates depends on the method of condensate formation. Phase transitions between these structures can be induced by thermal treatment. We conclude from these facts that with polyamine-DNA condensates metastable structures are of importance.

INTRODUCTION

In the last few years the structure of polyamine-nucleic acids complexes has been investigated by several authors (1-9). The results obtained from these relatively simple systems are important, on the one hand, for the elucidation of the function of the polyamines and for the understanding of more complicated protein-nucleic acid interactions, on the other.

The following essential findings can be summarized. The positively charged side chains of the polyamines neutralize the negatively charged phosphate groups of the polynucleotide chains. The formation of polyamine-DNA complexes does not lead to significant alterations of the B-conformation of the DNA (8). However, there remain some open questions. For instance, is it sufficient for condensation that the charges of the single DNA molecules are compensated, or are cross-

linking polyamine molecules between different DNA molecules necessary? We have found that, depending on the ionic strength, there can exist two different spermine-DNA condensates with different supramolecular structures (8). Both types reveal characteristic but extremely different X-ray diffractograms and CD spectra, respectively. We called them type I and type II condensates.

The aim of our experiments is to answer the following questions:

1. Are the type I and type II structures at the different ionic strength thermodynamic equilibrium structures or are they metastable structures?
2. Can both structures coexist?
3. Is it possible to convert the structure of the one type into the structure of the other type?

We think that from the physical point of view the results are of interest for understanding the intricate methods of the reconstitution of protein-nucleic acid systems.

MATERIALS AND METHODS

Spermine-DNA complexes were prepared by mixing sonicated calf thymus DNA and spermine-HCl (FERAK Berlin) solutions in 0.3 M NaCl, pH 7, as previously described (8). From this solution condensed complexes were produced by decreasing the NaCl concentration until turbidity appeared (< 0.2 M NaCl). The rate of the decrease was varied. We used three different rates:

1. Dialysis through polyacrylamide gel according to ZEPPEZAUER (10). The solution was dialyzed against 0.075 M NaCl during one week at 4°C in a refrigerator.
2. Slow dilution. The solution (10 ml) was diluted by adding bidistilled water dropwise at a rate of ten drops (approx. 0.4 ml) per minute under stirring.
3. Rapid dilution. Appropriate volumes of solution and bidistilled water were rapidly mixed under vigorous stirring to reach the desired ionic strength.

On the other hand NaCl concentration was stepwise raised from

0.1 M NaCl and 0.15 M NaCl, respectively, to 0.3 M NaCl by addition of small portions of 4 M NaCl, pH 7, to the turbid solutions (ca. 6 ml) of the condensed complexes under stirring.

CD measurements and CD-melting experiments were performed with a dichrographe CD 185 (ROUSSELL-JOUAN) equipped with a thermostated cell holder. Molecular ellipticities $[\theta]$ were calculated on the basis of a mean residue weight of 330 for a nucleotide. For a better clearness of the CD results we have demonstrated in some cases the dependence of the first CD extremum in the longer wave-length region on the ionic strength or on the temperature, respectively. This extremum we term $[\theta]_{max1}$.

The majority of the CD-melting curves have been recorded only partially in the region of $[\theta]_{max1}$. There were hints that during the time of a full recording the condensates could be damaged by the measuring radiation.

Turbidity measurements were performed with a spectrophotometer ACTA CV (BECKMAN) at 400 nm and a path length of 1 cm.

The X-ray scattering curves in the small-angle and wide-angle region were recorded by means of an automatic diffractometer with four scatter slits. Experimental details and evaluation of the data by a computer program were previously described (8).

RESULTS

As emphasized in the introduction, we have found two different types of spermine-DNA condensates at different ionic strengths (8). To make sure whether ionic strength alone is responsible for forming the one or the other type of condensates or whether velocity of condensation is of influence too, we have done these experiments, the results of which are shown in figure 1. It is obvious that slow condensation leads to condensates having a -Psi CD spectrum, while rapid condensation builds up condensates with a +Psi CD spectrum, regardless of the adjusted ionic strength.

We were also interested in the behaviour of the conden-

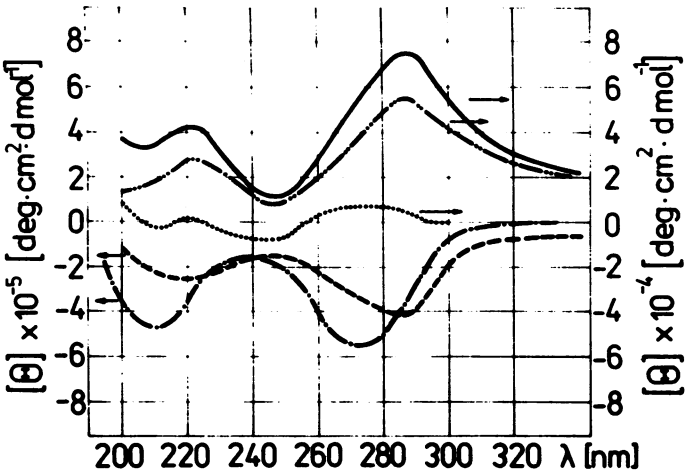

Figure 1: CD spectra of spermine-DNA complexes in dependence on the ionic strength and the velocity of condensing. ••• in 0.3 M NaCl (no turbidity); ---•--- in 0.15 M NaCl (rapid dilution); ___ in 0.075 M NaCl (rapid dilution); -•- in 0.15 M NaCl (slow dilution); - - - in 0.075 M NaCl (dialysis). DNA concentration of stock solution 3 mg/ml; DNA-P:spermine-HCl = 1:1 (mol/mol); path-lengths: 0.1 and 0.2 mm, resp..

sates at stepwise increase in the ionic strength. Especially we were interested in the question whether transitions from one type into the other exist. Increasing the ionic strength in the medium of the condensates having a +Psi CD spectrum at 0.1 M NaCl leads to a transition into such with a -Psi CD spectrum within a range of 0.14 M to 0.15 M NaCl. Further increase of the NaCl concentration causes, beginning at 0.16 M NaCl, a conservative spectrum which is identical with that of the initial solution at 0.3 M NaCl. The turbidity decreases continuously (figure 2). Condensates exhibiting a -Psi spectrum at about 0.15 M NaCl return to a conservative spectrum at a somewhat higher ionic strength without an intermediate transition into the +Psi type (figure 3).

The stability of the condensates was examined by melting experiments. An increase of the temperature of a +Psi type condensate causes enhancement of $[\Theta]_{max1}$; this could arise

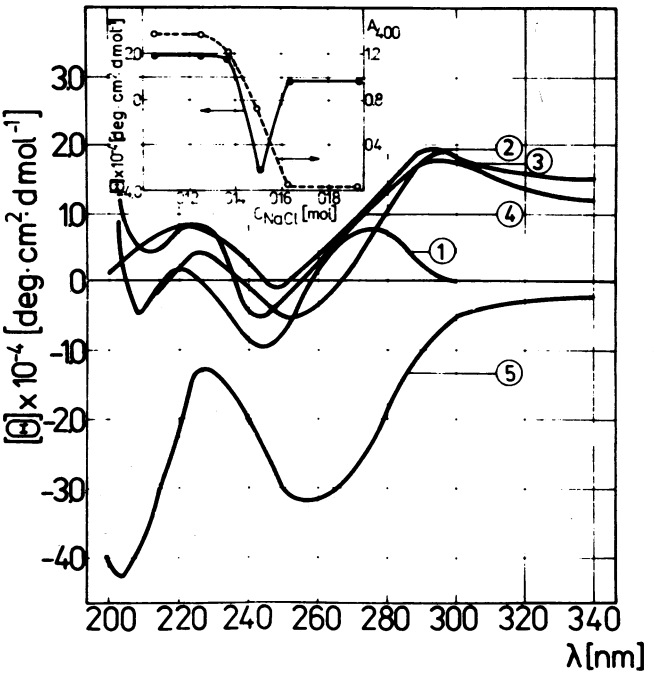

Figure 2: CD spectra of a +Psi spermine-DNA complex in dependence on increasing NaCl concentrations. 1: in 0.3 M NaCl (no turbidity); 2: rapidly diluted to 0.1 M NaCl; 3: increased to 0.12 M NaCl; 4: increased to 0.14 M NaCl; 5: increased to 0.15 M NaCl. Further increase leads to spectra identical with 1 (see inset). Path lengths: 0.2 mm. Inset: Dependence of $[\Theta]_{max\ 1}$ (——) and of turbidity (- - -) on NaCl concentration.

from light scattering effects of the solution. A transition from one type into the other does not occur (figure 4). However, a -Psi type condensate exhibits at first a blue shift of $[\Theta]_{max1}$. At 50°C a sharp transition into the +Psi type takes place, followed by a transition into a -Psi type at 75°C. Further increase of the temperature causes transition into a -Psi type again, showing comparatively small ellipticities (figure 5).

The results of the small-angle and wide-angle X-ray measurements are as follows: The condensates obtained by rapid dilution exhibit a type I X-ray diagram with a sharp

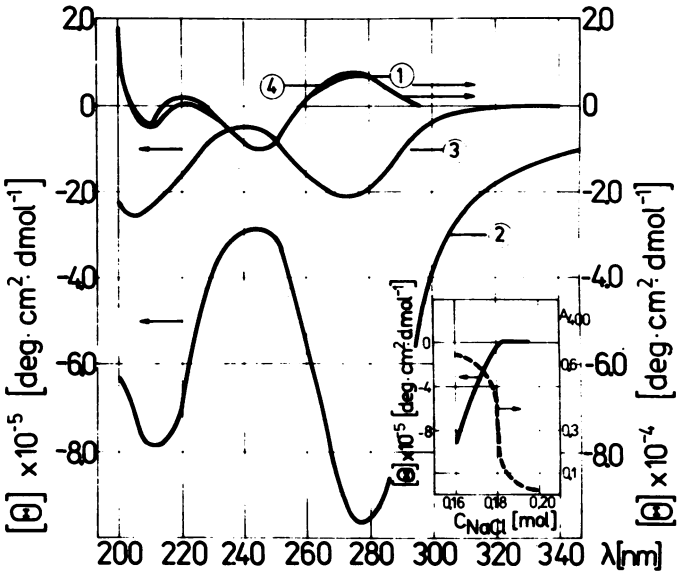

Figure 3: CD spectra of a -Psi spermine-DNA complex in dependence on increasing NaCl concentrations. 1: in 0.3 M NaCl (no turbidity); 2: diluted slowly to 0.16 M NaCl; 3: increased to 0.18 M NaCl; 4: increased to 0.19 M NaCl. Path lengths: 0.2 mm.
Inset: Dependence of $[\Theta]_{max1}$ (——) and of turbidity (---) on NaCl concentration.

reflex at a BRAGG value of d = 2.63 nm independent of the ionic strength (figure 6). The profile of the reflex is asymmetric. The intensity falls slowly to small scattering angles and rapidly to large scattering angles. The asymmetry is real and not an effect of a slit height smearing. The flanks of the reflex are superimposed by a fine-structure in form of ripples. Slow dilution leads to a diffraction diagram of type II in the region between 0.15 and 0.075 M NaCl, also independent of the ionic strength. This diagram shows a shoulder at a BRAGG distance of d = 3.27 nm. The shoulder is more pronounced at dilution by dialysis (IIa in figure 6) than at slow dilution by dropwise addition of water. No fine structure of the X-ray diffractogram can be observed. In the region of $2\Theta > 6^{\circ}$ the type I and type II diffractograms

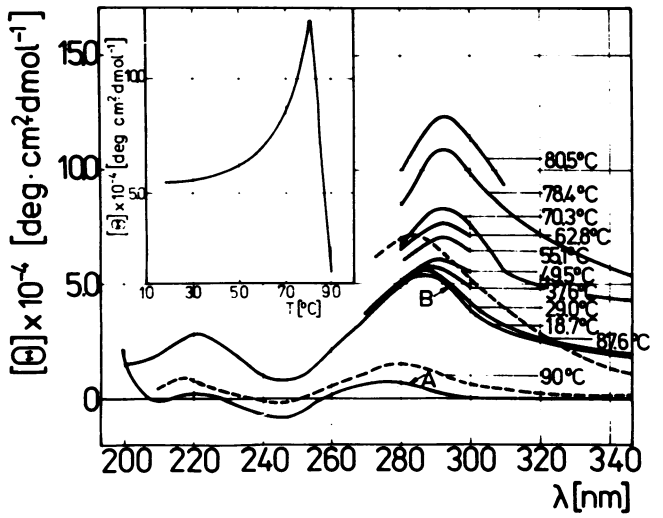

Figure 4: CD spectra, resp. parts of the spectra of a +Psi spermine-DNA complex in dependence on the temperature (a solution in 0.3 M NaCl, curve A, was rapidly diluted to 0.15 M NaCl, curve B, and then heated).
Inset: Dependence of $[\Theta]_{max1}$ on the temperature.

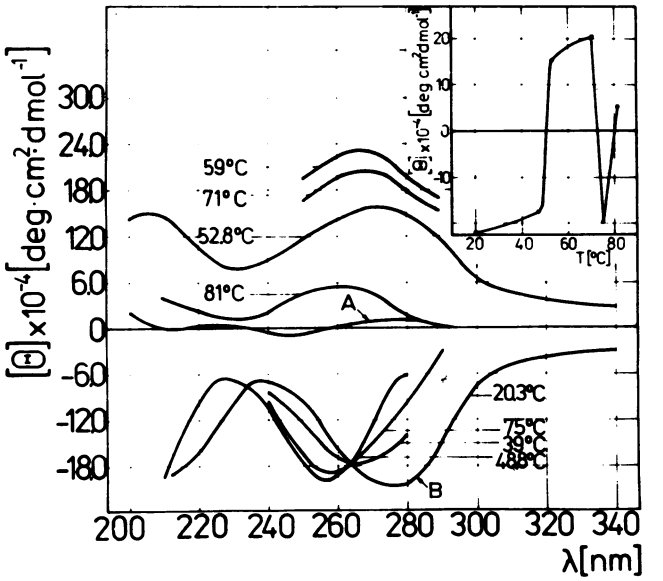

Figure 5: CD spectra, resp. parts of the spectra of a -Psi spermine-DNA complex in dependence on the temperature (a solution in 0.3 M NaCl, curve A, was slowly diluted to 0.16 M NaCl, curve B, and then heated).
Inset: Dependence of $[\Theta]_{max1}$ in dependence on the temperature.

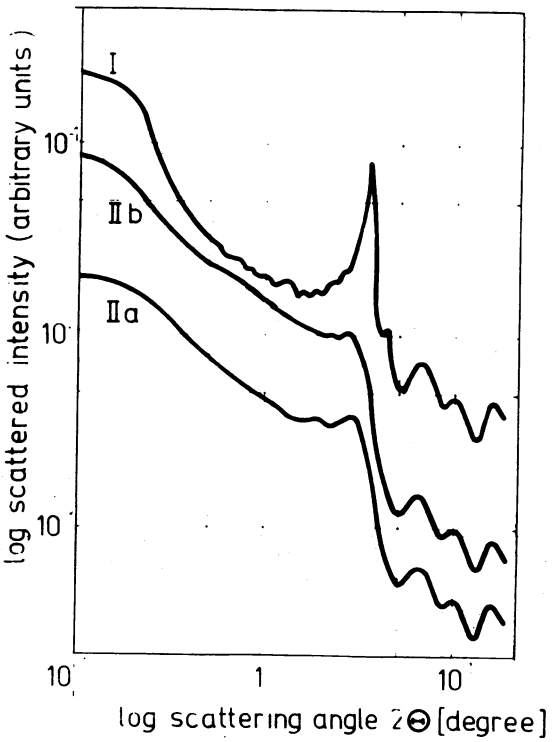

Figure 6: Small-angle and wide-angle X-ray scattering diagrams of spermine-DNA complexes in dependence on the ionic strength and the velocity of condensing. I: in 0.075 M NaCl (rapid dilution); IIa: in 0.075 M NaCl (dialysis); IIb: in 0.15 M NaCl (slow dilution).

exhibit no significant differences.

DISCUSSION

Our results are summarized in figure 7. The left ordinate represents the NaCl concentration. The direction and the velocity for reaching the indicated NaCl concentrations, i.e. rapid and slow dilution and dialysis, respectively, is symbolized by different arrows. The squares represent the samples examined.

First we discuss the influence of decreasing ionic strengths upon the structure of the complexes. At 0.3 M NaCl

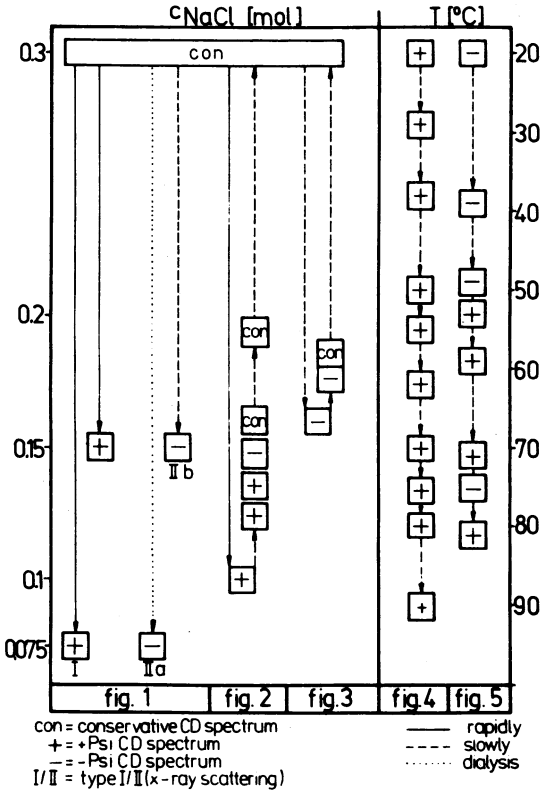

Figure 7: Summarized representation of results of the CD and X-ray measurements with the spermine-DNA complexes.

the solution reveals a conservative CD spectrum and no turbidity. There is no reason to assume a condensed state of DNA. Below 0.15 M NaCl suspensions are formed with non-conservative CD spectra, turbidity and changed diffraction patterns. This indicates condensed spermine-DNA phases (8). From the type of the CD spectra (figure 1) and the diffraction diagrams (figure 6) it is obvious, that there exist two different types (phases) of condensates. The +Psi type (CD) corresponds to the type I (X-ray) and the -Psi type to the type II, respectively. Both types have identical wide-angle X-ray scattering curves (figure 6) which are identical with the wide-

angle scattering curve of DNA in solution (8,12). The curves indicate a slightly modified B-form of DNA. However, the supramolecular structures of the two types are very different. The type I diagram is very similar to that of Na-DNA condensed by ethanol (11,12). Regarding the short-range order the X-ray diagram corresponds to side-by-side packing of DNA double helices. The ripples indicate that in addition to the short range order a long-range order exists also. From the type II diagram one can infer a special supercoiled or folded tertiary structure of DNA.

Secondly, the behaviour of both the type I (+Psi) and type II (-Psi) phases with increasing ionic strengths is of interest. In the region from 0.16 to 0.19 M NaCl the -Psi type condensate undergoes a co-operative transition to a solution having a conservative CD spectrum (figure 3). The decreasing turbidity indicates dissolution of the condensates. We cannot decide whether spermine-DNA complexes are present in the solution or whether the solution contains spermine- and DNA as separate components. The behaviour of the +Psi condensate is more complicated (figure 2). Between 0.075 and 0.13 M NaCl there is no influence of ionic strength on the CD spectrum and on turbidity. Between 0.13 and 0.16 M NaCl the turbidity decreases. On the contrary, the CD spectrum shows a phase transition between 0.14 and 0.15 M NaCl from the +Psi type to the -Psi type. Between 0.15 and 0.16 M NaCl dissolution of the condensates occurs. Above 0.17 M NaCl, DNA is in non-condensed state exhibiting a conservative CD spectrum. In summary, we find between 0.13 and 0.19 M NaCl one phase transition for the -Psi condensates and two transitions for the +Psi condensates.

Finally we examined the stability of both the +Psi and -Psi condensates in dependence on temperature (figures 4, 5 and 7). The +Psi condensates (figure 4) reveal an increase of positive $[\theta]_{max1}$ between 20°C and 80°C. Above 80°C a large decrease of the amplitude occurs and at 90°C the spectrum of non-condensed DNA is approximately recorded. At this temperature changes of the secondary structure (denaturation) cannot be excluded. The temperature dependence of the -Psi

condensates is more intricate (figure 5). Here three cooperative phase transitions take place at 51°C; 73°C and 78°C. A temperature dependent phase transition was also observed with polypeptide-DNA complexes (14) while a transition curve with several phase transitions similar to the curve shown in the inset of figure 5 is known from DNA condensed by polyethyleneglycol (PEG) in dependence on the PEG concentration (15). It should be emphasized that for experimental reasons it was not possible to identify the +Psi type with the X-ray type I and the -Psi type with the X-ray type II above room temperature. Therefore the correlation between the CD and X-ray types could be questioned at these elevated temperatures.

We would like to draw the following conclusions from this complicated behaviour of the spermine-DNA complexes:

1. At defined ionic strengths different structural types can coexist.

2. The thermodynamic state of a spermine-DNA system is not unambiguously determined by the external variables, such as temperature and ionic strength.

3. Besides the external variables, internal variables are also of importance. The state of the system is a function of the way in which the state is reached.

4. Measurements with one and the same spermine-DNA system, also carried out under the same external conditions, often lead to contradictory results. The reason of this are metastable states of the DNA molecules within the spermine-DNA system.

5. One set of data, for instance a CD spectrum, is not sufficient to describe the structure and consequently the properties of the system. So +Psi and -Psi condensates reveal similar CD spectra at about 60°C (figures 4 and 5). However, on further increase of the temperature they behave differently.

These conclusions have an experimental basis only for the examined spermine-DNA system. According to our experimental

results the assignment of the sign of the Psi-type CD spectra to the two structural types I and II, determined by X-ray measurements, is unambiguous for the spermine-DNA system. At this particular system the sign and only the sign is determined by the packing of the DNA within the condensates. The sign of the CD spectra is independent of the size of the condensates whereas the size influences the amplitudes (ellipticities) of the spectra. This was checked by experiments at different concentrations of DNA. This result must not be transferred to other DNA-polycation complexes. The CD spectra of condensed DNA are not unambiguously determined by the secondary structure of the DNA (11). For each particular system the assignment of the sign of the CD spectrum to the packing of the DNA must be decided by experiments. The general course of CD spectra of condensed DNA is determined in a very intricate manner by the supramolecular structures of the DNA within the condensed particles. The size of the particles may be of influence too.

There are, however, indications that our conclusions are generally valid for polycation-DNA systems. In a previous paper we have demonstrated metastable structures with Na-DNA condensed by ethanol (16). Metastable structures have also been found with 16S-rRNA (17). This leads to the suggestion that metastability may be a general property of DNA associates. Therefore it seems to us that, in the future, it will not be enough to characterize biochemical and biophysical experiments by the external thermodynamic variables alone, because reproducibility might not be guaranteed.

REFERENCES

1 Gosule, L. C. and Schellman, J. A. (1976) Nature 259, 333-335
2 Rubin, R. L. (1977) J. Bacteriol. 129, 916-925
3 Osland, A. and Kleppe, K. (1977) Nucl. Acids Res. 4, 685-695
4 Eickbush, Th. H. and Moudrianakis, E. N. (1978) Cell 13, 295-306
5 Gosule, L. C. and Schellman, J. A. (1978) J. Mol. Biol. 121, 311-326
6 Chattoraj, D. K., Gosule, L. C. and Schellman, J. A. (1978) J. Mol. Biol. 121, 327-337

7 Skuridin, S. G., Kadykov, V. A., Shashkov, V. S., Evdokimov, Yu. M. and Varshavsky, Ya. M. (1978) Mol. Biol. 12, 413-419
8 Damaschun, H., Damaschun, G., Becker, M., Buder, E., Misselwitz, R. and Zirwer, D. (1978) Nucl. Acids Res. 5, 3801-3809
9 Kawashima, S. and Ando, T. (1978) J. Biochem. 84, 343-350
10 Zeppezauer, M., Eklund, H. and Zeppezauer, E. S. (1968) Arch. Biochem. Biophys. 126, 564-573
11 Damaschun, H., Damaschun, G., Becker, M., Buder, E., Misselwitz, R. and Zirwer, D. (1978) Acta biol. med. germ. 37, 569-576
12 Giannoni, G. F., Padden, F. and Keith, H. D. (1969) Proc. Natl. Acad. Sci. U.S.A. 62, 964-971
13 Lerman, L. S. (1971) Proc. Natl. Acad. Sci. U.S.A. (1971) 68, 1886-1890
14 Krylov, A. S., Gurski, G. W., Kondrateva, N. O., Maryash, L. J., Poletayev, A. J. and Shibnev, W. A. (1978) Mol. Biol. 12, 297-307
15 Evdokimov, Yu. M. and Lortkipanidze, G. B. (1978) Dokl. Acad. Nauk SSSR 241, 1454-1457
16 Damaschun, G., Becker, M., Buder, E., Damaschun, H., Misselwitz, R. and Zirwer, D. (1978) studia biophysica 70, 205-212
17 Hochkeppel, H.-K. and Craven, G. R. (1977) J. Mol. Biol. 113, 623-634