Fluorescence study of secondary structure of DNA within bacteriophage λ

M.A. Shurdov and S.V. Kiyanov

All-Union Institute of Molecular Biology, Kol'tsovo Settlement, Novosibirsk Region, Novosibirsk District, 633159, USSR

Received 11 May 1983

Bromoacetaldehyde (BAA) was used to study the secondary structure of DNA in λ-phage particles. It was determined that about 1% of the adenines in the intraphage λ-DNA reacts readily with BAA, thus, they are placed in DNA sites with disturbed complementary interactions. These adenines are close to the tryptophan residues of the phage protein. Fluorescence emission of εA in the intraphage DNA is dramatically quenched. This, apparently, indicates the interaction between εA and Trp- and/or Tyr- and/or Met-residues of phage protein.

Intraphage DNA *Bromoacetaldehyde* *Secondary structure of DNA* *Phage λ* Bacteriophage λ

1. INTRODUCTION

Using IR- and Raman spectroscophy [1–3] and X-ray diffraction [4,5] it has been established that the overwhelming part of DNA within bacteriophages has the B-form. As yet, however, it remains obscure, whether all intraphage DNA has the double-stranded B-form or whether some part of the DNA bases is not involved in complementary interactions [5]. Here, bromoacetaldehyde (BAA) was used to study the secondary structure of DNA in λ phage.

BAA is known to react with mispaired DNA bases. The product of reaction of adenine with BAA, ethenoadenine (εA), has a high fluorescence emission, therefore BAA may be used to detect small quantities of denaturated DNA. The optical properties of εA are sensitive to nucleotide and protein surroundings. This makes it possible to use ethenoderivatives for investigations of polynucleotides and model nucleoproteins structures [7,8].

Abbreviations: εA, ethenoadenine(s), $1,N^6$-ethenoadenine(s); εAMP, ethenoadenosine 5′-phosphate, $1,N^6$-ethenoadenosine 5′-phosphate

2. MATERIALS AND METHODS

The preparation and purification of the bacteriophage λCI857 was performed as in [14]. The phage preparations obtained were kept in 10 mM Tris–HCl (pH 7.4), 100 mM NaCl, 10 mM $MgCl_2$. BAA was synthesized by G. Harkina and V. Petrenko as recommended in [6].

To modify intraphage DNA, phage (0.3 mg/ml) was incubated in 100 mM sodium cacodylate (pH 6.5), 100 mM NaCl, 10 mM $MgCl_2$ plus 39 mM BAA at 37°C. Aliquots (0.1 ml) of this mixture, taken during reaction, were diluted 5-times with cold 10 mM Tris–HCl (pH 7.4), 10 mM NaCl, 1 mM $MgCl_2$ to terminate the reaction and then were dialysed at 5°C against the same buffer to remove the unreacted BAA.

Fresh phenol-extracted λ-DNA was modified under the same conditions as the λ-phage.

The intactness of the phage particles was monitored by the electron microscopy. Samples for electron microscopy were prepared with 1% formaldehyde to prevent the phage destruction during phosphostungstic acid treatment.

To determine the εA quantity, the modified

Published by Elsevier Science Publishers B.V.
00145793/83/$3.00

DNA was isolated from the phage (when whole phage particles were treated with BAA) and digested by DNase I and snake venom phosphodiesterase. The fluorescence intensity of digested DNA was measured and compared to ϵAMP fluorescence intensity used as a standard.

Fluorescence of ϵA was excited at 310 nm and measured at 410 nm. Technical fluorescence excitation and emission spectra were measured on a Hitachi Perkin-Elmer MPF-44B fluorescence spectrophotometer. The relative quantum yields of fluorescence were estimated from the intensity of fluorescence at 410 nm, taking into account that the molar extinction coefficient at 310 nm for ϵAMP is 1.4-times more than the extinction coefficient for ϵA in DNA [6,12].

3. RESULTS AND DISCUSSION

3.1. *Kinetics of modification*

As seen from fig.1, about 1% of adenines in the intraphage DNA react with BAA very readily (~15-times faster than adenines in free DNA). Then modification slows down and resumes only after 30 h with low rates. After this time the modification is accompanied by marked phage destruction and stops in 130–140 h. In this case the degree of modification reaches 10–12% of the total adenine content and 40–50% of phage particles are destroyed. The lability of the phage particles during the prolonged modification enables determination of the maximal percentage of modified adenines in the intraphage DNA. However, the formation of ϵA in the intraphage DNA after the 30-h lag-period is unlikely to be attributable to destruction of phage particles since the free DNA is far less modified.

Also, specific rapid modification of about 1% of intraphage adenines indicates that these bases have amidine fragments; i.e., these adenines are not involved in complementary interactions.

The occurrence of a lag-period and subsequent modification are in good agreement with the data in [9] on the deamination of DNA in the S_d phage particles by nitrous acid. They established that quite a number of unpaired bases (adenines and cytosines) of the intraphage DNA had amino groups blocked by a noncomplementary mechanism. Also, the bases were assumed to be blocked by DNA–protein interactions. Nevertheless, taking into account the accuracy of the method used, the authors did not preclude the possibility of the presence of a small amount of DNA bases with non H-bonded amino groups in a phage.

3.2. *The relative quantum yields of fluorescence (RQY)*

The changes in RQY for ϵA in the modified intraphage DNA and in DNA1 (DNA, isolated from the phage after reaction with BAA) are reproduced in fig.2 as a function of the percentage of modified

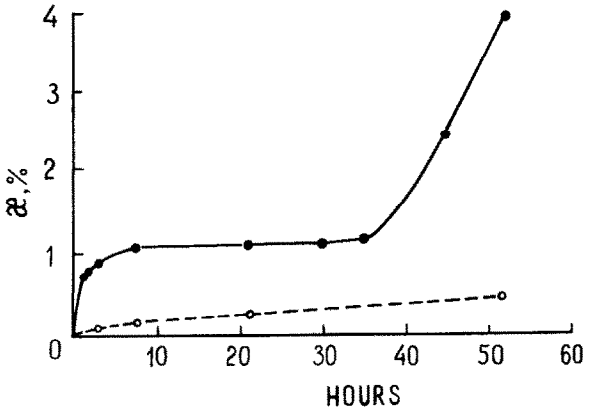

Fig.1. Time course of adenine modification by BAA for intraphage DNA (a solid curve) and for free DNA (a dotted curve). æ = the quantity of ethenoadenines in the whole number of DNA adenines as a percentage.

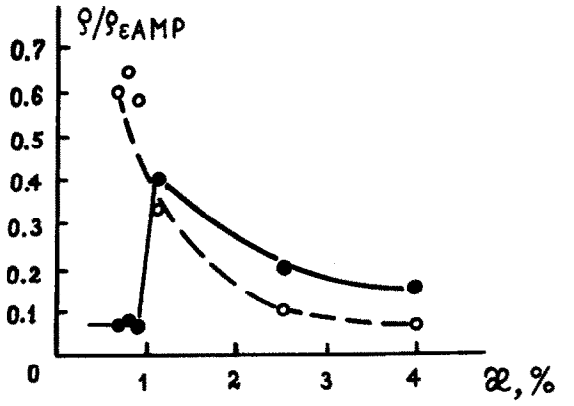

Fig.2. The change in the relative quantum yields: (—●—) for ϵA in the modified intraphage DNA; (---○---) for ϵA in DNA1 (DNA, modified in the phage and then extracted into the solution) as a function of the percentage of adenine modified.

adenines. At æ < 1% RQY for DNA1 is close to the value 0.6–0.7. This value is characteristic for poly(A,ϵA) at a low percentage of ϵA [7]. RQY for some heterogeneous polynucleotides containing ϵA are known to be significantly less and to vary about the mean value 0.1 [7]. This suggests that the intraphage DNA sites with disturbed complementary interactions of the bases have the adjacent adenine residues in the same DNA chain.

Further modification causes the RQY for ϵA in DNA1 to fall sharply (fig.2, æ ⩾ 1%). This may be due to further modification of adenines in sites –A–ϵA–A–, that gives rise to structures: –A–ϵA–ϵA– and then –ϵA–ϵA–ϵA–, wherein RQY can be estimated as 0.1 and 0.02, respectively [7]. Using the formalism developed in earlier papers [7,10] one can estimate that probability of ϵA formation out of the sites of initial modification of the intraphage DNA does not exceed 0.01.

At a low degree of modification (æ < 1%) RQY for ϵA in the modified intraphage DNA is 10-times lower than the corresponding value for ϵA in DNA1 (fig.2). The possible reason of this quenching may be the ϵA–protein interactions. Indeed, model experiments indicate that L-Met, L-Trp and L-Tyr quench the fluorescence of ϵAMP in solution [11]. Also, a Trp-residue was found to cause the strongest quenching because of the effective formation of a ground–state complex with an adenine residue in model compounds [12]. As yet there is no information about the interactions between these amino acids and DNA in phages.

Further modification (æ ⩾ 1%) leads to the sharp increase of RQY for ϵA in the intraphage DNA. This increase can be ascribed to such a change of the structure of the phage nucleoprotein that makes protein unable to quench the fluorescence of ϵA.

3.3. *Fluorescence excitation spectra*

Fluorescence excitation spectrum of ϵA in the modified intraphage DNA has an intensive band at 285 nm which coincides with the fluorescence excitation spectrum of Trp-residues in phage (fig.3,α). This indicates the effective excitation energy migration from Trp-residues to ϵA at low degrees of adenine modification and, therefore, the proximity of ϵA and Trp-residues in phage particles. It should be noted, that the DNA release from the phage causes the disappearance of the

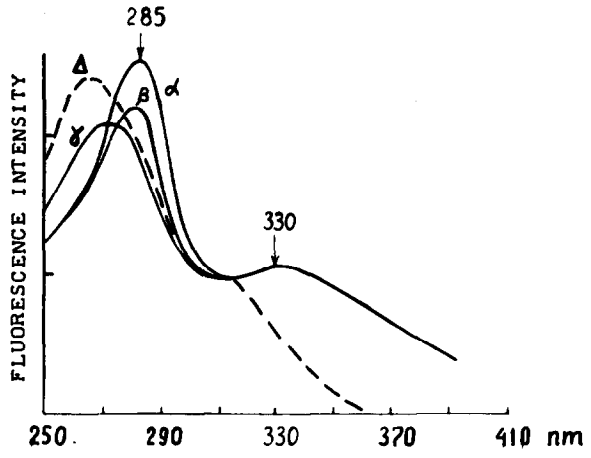

Fig.3. Fluorescence excitation spectra for ϵA in the intraphage DNA at æ = 0.9% (α), æ = 1.1% (β) and æ = 4% (γ); for ϵA in DNA1 at æ = 0.9% (Δ). An intensive band at 285 nm coincides with the fluorescence excitation spectrum of Trp-residues in the λ phage and indicates the proximity of these residues and intraphage DNA ethenoadenines.

band at 285 nm and an increase of the band at 260–270 nm. The latter band is likely due to excitation energy migration to ϵA from adjacent DNA bases (fig.3,Δ).

At æ ⩾ 1% energy migration from Trp-residues to ϵA decreases. This may be due to both the change in arrangement of initial ϵA and Trp in phage (fig.3,β) and the formation of new ϵA which are more distant from Trp in a phage (fig.3,γ).

The proper fluorescence excitation maximum at 310 nm for ϵA in DNA1 and in the free DNA is red-shifted to 330 nm in the intraphage DNA (fig.3). Such red-shifts cannot be caused only by the ϵA surrounding polarity change in the phage head as compared to the solution or dehydration of DNA bases in phage. Indeed, it is known for ϵAMP that even in absolute dioxan solution the maximum is red-shifted by only 9 nm [15], and ϵAMP dehydration in 6 M LiCl does not shift the maximum at all [8]. Such a large red-shift may be caused by the formation of a charge transfer complex (CTC) between ϵA and amino acid residues of phage protein analogous to CTC in the frozen solution of amino acids and adenine [13].

ACKNOWLEDGEMENTS

We wish to thank Dr N. Bozhko for supplying us phage λCI857 and G. Harkina and Dr V. Petrenko for bromoacetaldehyde preparation and useful discussion. We are grateful to Dr A. Razjivin for the friendly support we felt during our work.

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