PHOTOSENSITIZED SPLITTING OF THYMINE DIMERS IN DNA BY GENE 32 PROTEIN FROM PHAGE T 4

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Received April 9, 1976

Summary

The binding of denatured DNA to the protein coded by gene 32 of phage T 4 is accompanied by a quenching of the fluorescence of the protein tryptophyl residues. Gene 32 protein also binds to UV-irradiated DNA and photosensitizes the splitting of thymine dimers. Thymine bases are regenerated by this photosensitized reaction both in double stranded and in heat denatured DNA. No photosensitized splitting of thymine dimers is observed when the complex formed by gene 32 protein with UV-irradiated DNA is dissociated at high ionic strength. These results are discussed with respect to the possible stacking interaction of tryptophyl residues of gene 32 protein with bases in single stranded DNA.

Introduction

We have previously shown that a simple tripeptide such as L-lysyl-L-tryptophyl-α-L-lysine (Lys-Trp-Lys) is able to discriminate between single stranded and double stranded regions in DNA which has been submitted to UV irradiation[1,2]. This peptide binds more strongly to single stranded regions because the stacking of the tryptophyl aromatic ring with nucleic acid bases is favored in these regions[3,4].

One of the main damages formed in DNA following UV irradiation is represented by pyrimidine dimers among which thymine-containing dimers are the most important[5]. When the complex formed by Lys-Trp-Lys and UV irradiated DNA was further submitted to UV irradiation, thymine dimers were split and thymine bases regenerated[1,2]. This photosensitized splitting appears to involve electron transfer from the indole ring to the dimers. It requires a close contact between these two components of the peptide-nucleic acid complex and therefore a stacking of the indole ring of the tryptophyl residue of the peptide with the bases of the nucleic acid.

If photosensitized splitting of thymine dimers could be demonstrated in a protein-nucleic acid complex this would lend strong support to the involvment of tryptophyl residues of the protein in stacking interactions with the bases. This should be especially true for proteins which recognize single stranded DNAs since the presence of thymine dimers in such a DNA would not be expected to affect markedly protein binding. This is probably not true in the case of double stranded DNA where local base unpairing due to thymine dimer formation might considerably modify the interaction with the protein.

As a model protein we have chosen the protein coded by gene 32 of phage T 4[6, 7]. This protein has been shown to be required for the replication of the phage DNA. It binds strongly and cooperatively to single stranded DNA. We have studied the binding of gene 32 protein to native, heat denatured and UV irradiated DNA by fluorescence spectroscopy. Then the photosensitized splitting of thymine dimers in UV irradiated DNA was investigated before and after heat denaturation of the DNA.

Materials and methods

^{3}H-thymine-labeled DNA was prepared from E. coli B_3T^- strain. The protein coded by gene 32 of phage T 4 was obtained according to the procedure described by Alberts and Frey[6] with an additional step of column chromatography on hydroxyapatite[8].

Fluorescence measurements were carried out at 15°C with a Jobin-Yvon spectrofluorometer modified as described by Brun et al.[3] All binding experiments were carried out at pH 7 in a buffer containing 10^{-2} M NaCl, 10^{-2} M Na cacodylate and 2 x 10^{-3} MEDTA (Buffer A). Ionic strength was varied by adding concentrated NaCl.

UV irradiation was performed with a HBO 200 W lamp filtered through water and a MTO H 325a filter which cuts light off below 250 nm and above 390 nm. Light intensity was measured with a Kipp and Zonen thermopile. The buffer used for UV irradiation (buffer B) contained 10^{-2} M NaCl and 10^{-2} M Na cacodylate at pH 7 (without EDTA).

For the determination of thymine dimer concentration the irradiated samples were hydrolyzed in formic acid at 175°C for 30 minutes as described by Setlow and Carrier[9]. The hydrolyzed material was then sub-

jected to descending paper chromatography in acetic acid-n butanol-water (12:80:30 v/v). Strips from the chromatograms were then counted in a Beckman scintillation counter.

Results

1) Binding of gene 32 protein to denatured, native and UV irradiated DNAs

When excited at 290 nm gene 32 protein emits a fluorescence which is nearly exclusively due to its tryptophyl residues. Adding single stranded DNA leads to a quenching of this fluorescence due to complex formation. At low ionic strength a plateau corresponding to about 40 % quenching is obtained and the binding is strong enough to permit the determination of the site size by extrapolation of the initial part of the binding curve (Figure 1). It can be calculated that the binding site of gene 32 protein on single stranded DNA covers about 7 nucleotides. When the ionic strength increases the binding constant decreases. As can be seen on figure 1 addition of NaCl to a protein-DNA mixture at low ionic strength (buffer A) leads to an increase in fluorescence intensity due to complex dissociation. The fluorescence quantum

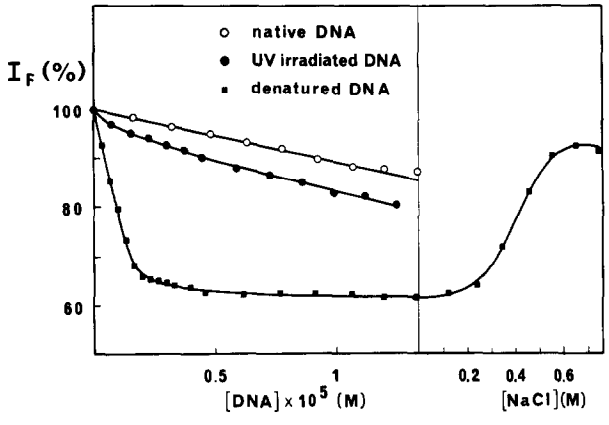

Figure 1 : Fluorescence intensity changes induced by the binding of gene 32 protein to native (•), UV irradiated (x) and heat denatured DNA (o). Protein concentration was 2.6×10^{-7} M. The UV irradiated DNA sample contained 16 % of its thymines converted into dimers.

In the right part of the figure is shown the effect of ionic strength on the fluorescence behavior of the complex formed by gene 32 protein with denatured DNA (It should be noted that the fluorescence quantum yield of gene 32 protein decreases by about 10 % when NaCl concentration increases from 0.01 to 0.7 M)

yield of gene 32 protein slightly decreases when ionic strength increases. When NaCl concentration is higher than 0.6 M the fluorescence intensity of the protein-DNA mixture reaches the value corresponding to the free protein indicating complete complex dissociation under these experimental conditions.

In contrast to denatured DNA, native DNA induces only a slight decrease of gene 32 protein fluorescence (see figure 1). This is due to weak binding and not to a smaller effect of binding on the protein fluorescence as demonstrated by a competition experiment in which native DNA was added to the gene 32 protein - denatured DNA complex. No increase in fluorescence was detected indicating that native DNA did not compete with denatured DNA for the protein (data not shown).

When native DNA is irradiated with UV light, several damages are created in the nucleic acid among which pyrimidine dimers are by far the most important[5]. The formation of such dimers with a cyclobutyl ring leads to a local distortion of the DNA helix and results in local unpairing of bases. The size of the corresponding unpaired region has been estimated to be around 4 base pairs[10]. However it should be noted that UV damages other than pyrimidine dimers markedly contribute to the destabilization of DNA[11].

The fluorescence of gene 32 protein is quenched by UV irradiated DNA to a larger extent than by native DNA indicating a more efficient binding (Figure 1). When UV irradiated DNA is denatured by heating, the quenching of gene 32 protein fluorescence is indistinguishable from that induced by denatured DNA which has not been UV irradiated.

2) Photosensitized splitting of thymine dimers in DNA by gene 32 protein

Native E. coli DNA was first irradiated in buffer B as described in "Materials and Methods" until about 20 % of thymines were converted into dimers. This DNA was denatured by heating the sample to 100° and rapid cooling. Then gene 32 protein was added up to a concentration of 1 protein molecule per 5 phosphates. The protein-DNA complex thus formed was then subjected to UV light under the same irradiation conditions as those used for making thymine dimers in native DNA. The content of thymine dimers was determined after different periods of irradiation. The percentage of thymine dimers decreases very rapidly upon irradiation in the presence of gene 32 protein. About 62 % of the dimers could be split under our experimental condi-

tions (table 1). In the absence of the protein, the same DNA sample showed a slight increase (1-3 %) in thymine dimer content. If the mixture of gene 32 protein and denatured UV irradiated DNA was irradiated at high ionic strength (1 M NaCl) practically no splitting could be observed (table 1).

Table 1 : Percentage of thymine dimers split in UV irradiated DNA (6×10^{-6} M) following irradiation of its complex with gene 32 protein (1.2×10^{-6} M) in buffer B with NaCl concentration indicated. The complex was irradiated for 10 minutes corresponding to a total incident light intensity of 5×10^{5} J. m^{-2} (in the total wavelength range transmitted by the filter ; see "Materials and Methods"). The UV irradiated DNA sample contained 20 % of its thymines as dimers. The complex with gene 32 protein was formed either before (1st line) or after (2nd and 3rd lines) heat denaturation of the UV irradiated DNA.

	NaCl (M)	% split T<>T dimers
Native DNA	0.01	44.5
Denatured DNA	0.01	62.2
Denatured DNA	1.0	6.5

If the complex formed at low ionic strength was irradiated for long periods of time, the content of thymine dimers increased. This is due to a degradation of gene 32 protein by UV irradiation. Protein damage could be followed by the decrease of tryptophyl fluorescence. As irradiation proceeds, the tryptophyl residues of gene 32 protein are progressively destroyed and the efficiency of photosensitized splitting decreases resulting in an increase of thymine dimer concentration due to direct photolysis of DNA.

If gene 32 protein was added to a UV irradiated DNA sample which had not been heat denatured, irradiation of the mixture let to a photosensitized splitting of thymine dimers (table 1). The efficiency of the photosensitized reaction was however lower than with denatured DNA. This result was expected since binding of gene 32 protein to UV irradiated DNA is much stronger after this DNA has been denatured by heating (figure 1).

Discussion and Conclusion

The results presented above show that

i) gene 32 protein binds much more strongly to denatured than to native DNA as already published[6, 7]. One molecule of protein covers about 7 nucleotides in single stranded DNA.

ii) the fluorescence of the tryptophyl residues of this protein is quenched upon complex formation with denatured DNA. A similar result was recently observed upon binding of the E. coli unwinding protein to denatured DNA[12].

iii) gene 32 protein is able to photosensitize the splitting of thymine dimers produced in DNA by previous UV irradiation. This photosensitization occurs only if the protein is bound to UV irradiated DNA.

These results are quite similar to those previously obtained with the tripeptide Lys-Trp-Lys (except for the site size). We showed that the tryptophyl residue of this tripeptide was able to photosensitize the splitting of thymine dimers in UV irradiated DNA. This ability was related to the stacking of tryptophan with bases in the unpaired regions created around thymine dimers. This stacking interaction was responsible for the quenching of tryptophan fluorescence. The indole ring of tryptophan is known to be a good electron donor. Electron transfer from tryptophan to thymine dimer was proposed as the initial step of the photosensitized splitting reaction. It must be added that thymine dimers do react with solvated electrons. However solvated electrons which could be formed upon photoionization of the tryptophyl ring of Lys-Trp-Lys do not contribute significantly to thymine dimer splitting in peptide-DNA complexes since N_2O (which is a good electron scavenger) has no effect on the splitting reaction. Therefore the electron transfer step in the photosensitized splitting reaction requires a close proximity (good orbital overlap) between the tryptophan ring and the thymine dimer.

The results obtained with the complexes formed by gene 32 protein with UV irradiated DNA clearly show that at least one tryptophyl residue of the protein is certainly stacked with thymine dimers. The binding of gene 32 protein to denatured DNA does not depend markedly on whether the DNA has been submitted to UV irradiation or not (even when the irradiated DNA sample contains as much as 20 % of its thymines converted into dimers). Fluorescence quenching induced by binding is the same. Thus two observations (tryptophan fluorescence quenching and photosensitized splitting of thymine dimers) are good arguments for the involvment of tryptophyl residues of

gene 32 protein in complex formation with denatured DNA. A stacking interaction with nucleic bases is strongly suggested. A photosensitized splitting of thymine dimers also takes place in UV irradiated DNA which has not been denatured by heating. This result as well as fluorescence data show that gene 32 protein binds to the unpaired regions created in native DNA by UV irradiation and that tryptophyl residues (at least one) are involved in the binding.

It has recently been shown that the fluorescence of the unwinding protein from E. coli is also quenched when it binds to denatured DNA and single stranded polynucleotides.[12] Such a fluorescence quenching appears to be a general phenomenon in the complexes formed by proteins and nucleic acids at least when these nucleic acids are single stranded or contain single stranded regions. This is the case, e.g., for aminoacyl tRNA synthetases interacting with their cognate tRNAs. Several mechanisms can account for the fluorescence behavior of tryptophyl residues in protein-ligand complexes. However the generality of the quenching phenomenon observed when the ligand is a nucleic acid points out to the involvment of a specific mechanism, namely stacking interaction with bases. The results reported here show that this type of interaction is likely to be involved in the binding of the protein coded by gene 32 of phage T 4 to denatured DNA.

Acknowledgments : we wish to thank Mrs A. Chestier for her assistance in the purification of gene 32 protein.

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