CONVERSION OF NON-COVALENT INTERACTIONS IN NUCLEOPROTEINS INTO COVALENT BONDS: UV-INDUCED FORMATION OF POLYNUCLEOTIDE–PROTEIN CROSSLINKS IN BACTERIOPHAGE Sd VIRIONS *

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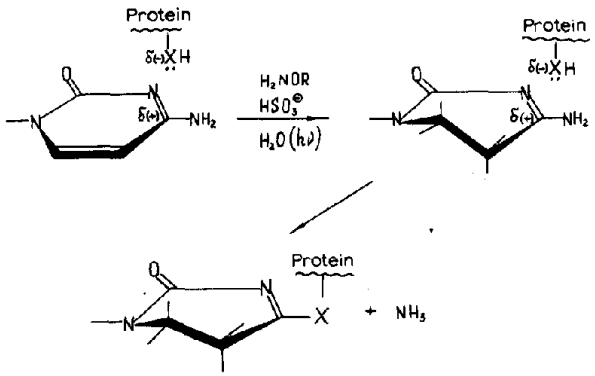
1. Introduction

The quarternary structure of nucleoproteins is stabilized by specific, cooperative, non-covalent interactions between polynucleotide and protein components. Conversion of these non-covalent interactions to covalent polynucleotide–protein crosslinks could be of great help in isolation and investigation of the structure and mutual orientation of the two interacting components of nucleoprotein. Understanding the mechanism of the formation of such links would also reveal the factors responsible for the specificity of interactions within the nucleoprotein.

Polynucleotide–protein links are well known to arise as the result of ultraviolet (UV) irradiation of viruses and cells (see, for example, [2–6]. Among the underlying reactions could be the interactions of the excited uracil and thymine nuclei with amino acid residues [7–14]. Strong nucleophilic agents are also known to induce crosslinks in viral nucleoproteins, involving cytosines driven out of the DNA double helix due to specific interactions with phage proteins [15–17] (see scheme 1).

The possibility of the latter reaction was strengthened by experiments with model mixtures [1,18]. Our results which are to be published elsewhere have demonstrated that the products of UV–irradiation of mixtures of cytidine with *O*–methylhydroxylamine or peptides, are formed (see the above scheme) by substitution of the exocyclic amino group in a saturated cytosine derivative – cytidine photohydrate (cf. [19]). These results imply that not only thymine and uracil but also cytosine nuclei involved in non-covalent interaction with proteins would be the subject of crosslinkage upon UV-irradiation of nucleoproteins, according to the above scheme.



Scheme 1.

The involvement of pyrimidine bases in higher structures results in the lowering of the rate of their reaction with nucleophilic agents by more than two orders of magnitude [15,20–22], but has a relatively small effect on the quantum yield of UV-induced hydration [23–25]. This fact as well as the possibility of covalent linkage in other types of polynucleotide–protein interactions makes the UV-induced crosslinks an important tool for the study of nucleoprotein structure.

On the basis of the above rationale we have studied

* Communication II of the series. The first paper of this series is [1].

the induction by UV of crosslinks in bacteriophage Sd; previously this phage had been used for the investigation of crosslinkage induced by nucleophilic agents [15–17].

The results presented below demonstrate that the linkage occurs only when a whole phage nucleoprotein is irradiated and that the binding of a single protein molecule is associated with photochemical damage to 0.3–0.03% of the nucleoside residues.

2. Materials and methods

Unlabelled and ^{35}S-labelled bacteriophage Sd purified by centrifugation in CsCl gradient and gel-filtration on Biogel P-300 [26–28]was a gift of Prof. T.I. Tikchonenko and Dr N.P. Kisseleva. Phage was disrupted by heating for 30 min at 70°C [15]. The phage concentration was determined as the DNA content which was assayed by the content of phosphorus. Intact or disrupted bacteriophage at 30–100 μg DNA per ml in 0.1 M NaCl was irradiated under a low-pressure mercury lamp in sealed quartz cuvettes at 1–2 mm solution thickness and 20°C.

After the irradiation, the phage suspension was diluted with 0.1 M NaCl to the final concentration of 30 μg DNA per ml and divided into aliquots. One series of the aliquots was heated to disrupt virions, and incubated further with 1 mg/ml pronase (Serva, nuclease-free) for 2 hr at 37°C. The viscosity of the solutions was measured in aliquots directly after irradiation, after heating and after incubation with pronase using an Ostwald viscosimeter. The results are presented in fig. 1.

Another series of the aliquots of irradiated and nonirradiated phage, after heating, was centrifuged in gradients of Cs_2SO_4 in an SW-50 rotor at 35000 rpm for 17 hr at 5°C.

The amount of [^{35}S]protein covalently bound to DNA was determined by phenol deproteinisation on thin-layer plates of DEAE cellulose [29]. Prior to application on the plate, the phage in an aliquot was disrupted by adding an equal volume of saturated SDS solution and heating at 70°C for 10 min.

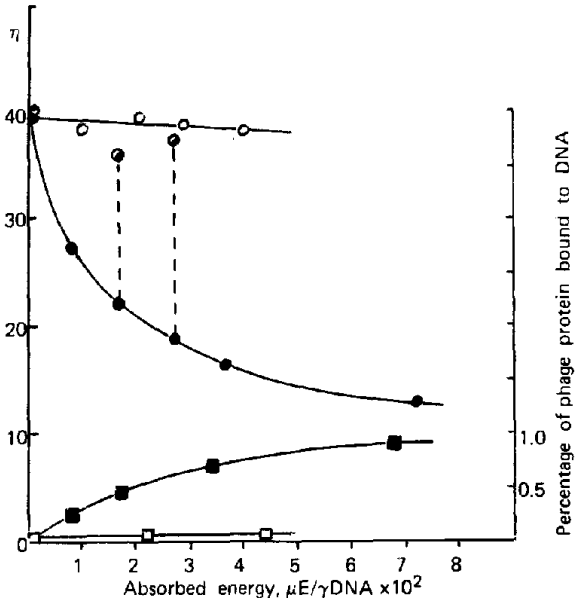


Fig. 1. The effect of UV-irradiation of bacteriophage Sd on the viscosity of the solutions of heat-disrupted virions (circles) and on the amount of [^{35}S] protein bound to DNA after phenol deproteinisation on plates (squares).

Open symbols, phage disrupted before irradiation; closed symbols, phage disrupted after irradiation; half-closed symbols, phage disrupted after irradiation and treated with pronase.

3. Results and discussion

Thermal disruption of phage virions results in the complete separation of DNA and proteins which is manifested as an increase of the solution viscosity [30]. In the case of UV-irradiated bacteriophage this increase diminishes with the absorbed energy (fig. 1). Several possibilities could account for this: (a) UV-induced DNA degradation; (b) stabilisation of the protein coat of the phage, e.g. due to protein–protein linkage; (c) polynucleotide–protein crosslinks.

As is evident from fig. 1, the irradiation of a mixture of DNA and phage proteins (preheated phage) under the same conditions has no effect on the vis-

cosity. Moreover, the pronase treatment of the irradiated and then disrupted phage results in the increase of the viscosity up to the level characteristic of free undegraded DNA (fig. 1). On the other hand, the formation of protein–protein links is virtually excluded under the conditions used [31]. Thus the decrease of the viscosity of the disrupted phage caused by UV-irradiation is due not to the degradation of DNA but rather to the induction of polynucleotide–protein crosslinks preventing the complete separation of proteins from the DNA.

The fact that the viscosity did not change when the mixture of phage proteins with double-helical DNA was irradiated (fig. 1, cf. [15]) implies that the formation of crosslinks requires a certain closeness and fixed mutual orientation of the reacting DNA and protein components, which is only possible within the phage nucleoprotein due to noncovalent polynucleotide–protein interactions.

UV-irradiation of phage also results in sharp changes of the caesium sulfate sedimentation pattern of the disrupted virions (fig. 2). After the irradiation, a significant fraction of the DNA and virtually all the protein are recovered from the gradient region with a buoyant density of about 1.34. Similar changes have been observed as the result of polynucleotide–protein crosslinkage induced by nucleophilic agents [16]. Obviously, neither these agents nor the UV-irradiation can covalently bind all coat protein to the DNA. Instead, one can suppose that free protein subunits strongly associate with those which have been crosslinked to polynucleotide.

To detach the associated subunits and to measure the amount of the protein covalently bound to DNA, we used a new rapid method, the detachment of unbound protein by phenol deproteinisation on plates with thin layers of DEAE-cellulose [29]. As is evident from fig. 1 the amount of bound protein increases with the absorbed energy.

No such changes were observed after irradiation of the mixture of phage proteins with DNA. Thus, this method also detects crosslinks only when intact virions are irradiated, which implies that a certain orientation of DNA and protein components is required for the linkage.

The comparison of UV-energy absorbed, the amount of bound protein and the composition of

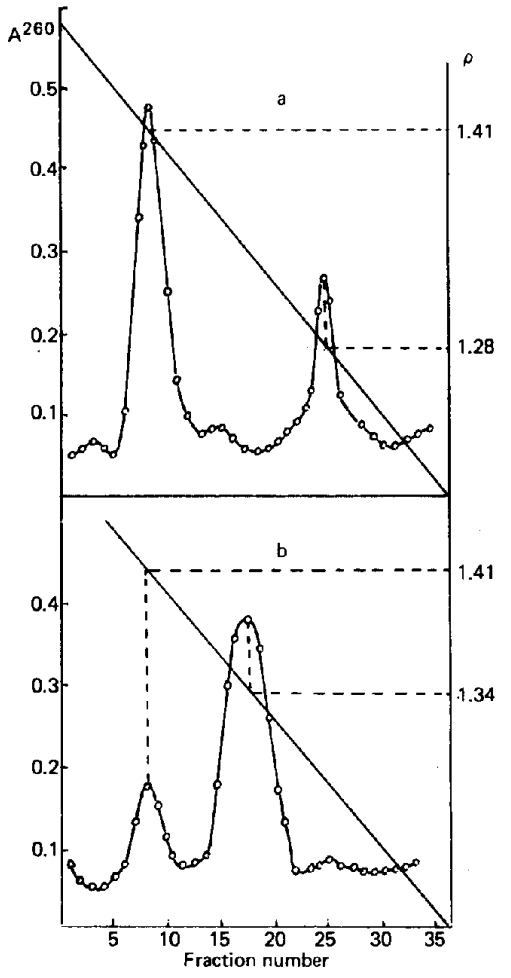


Fig. 2. The distribution in caesium sulfate gradients of heat disrupted phage Sd. (a) non-irradiated phage; (b) irradiated phage. Absorbed energy – 0.8×10^{-2} μE per μg DNA. The same pattern was obtained at 3×10^{-2} μE per μg DNA (data not shown).

phage virions allows one to estimate the efficiency of crosslinkage upon UV-irradiation. The molecular weight of bacteriophage Sd is about 1.1×10^8 of which protein constitutes some 54% [32]. The phage protein includes about 30 types of molecules of molecular weight ranging from 10^4 to 9×10^4 (Zintshenko, A.I. and Kisseleva, N.P. in preparation). One can see from fig. 1 that the absorbance of 3×10^{-2} microeinstein (μE) per μg DNA,

i.e. 1.5×10^6 quanta per virion, results in the binding of about 1% protein to DNA, that is 7–60 molecules per DNA complement. Hence the binding of a single protein molecule must require the absorption of $2 \times 10^4 - 2 \times 10^5$ quanta. The quantum yield of photochemical changes of pyrimidine moieties in DNA is about 3×10^{-3} [23,25,33,34]. Consequently, the binding of a single protein molecule to a DNA chain is accompanied by photochemical alteration of only 0.3%–0.03% of nucleoside moieties.

The linkage of DNA to protein affects the process of bacteriophage DNA injection into host cells [6, 35, 36] and, in eukaryotes, would be the cause of chromosome aberrations (cf. [37]). The repair of such links by photoreactivation or a dark repair system is unlikely. Hence the inactivation of viruses and cells by UV could be, to a large extent, due to the polynucleotide protein crosslinks, in spite of the fact that they are formed at a considerably lower rate than other types of photochemical lesions of nucleosides.

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